ORIGINAL SUBMISSION

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GRN 000625

nascent
HEALTH SCIENCES

February 1, 2016

Paulette Gaynor, Ph.D.
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food And Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Dear Dr. Gaynor:

In accordance with 21 CFR 170.36 (62 FR 18960; April 17, 1997), Nascent Health Sciences, LLC is hereby submitting notice of its determination based on scientific procedures that the use of pyrroloquinoline quinone (PQQ) disodium salt, as defined in the enclosed documents, is generally recognized as safe (GRAS) under specific conditions of use as an ingredient in multiple food categories, and that it is therefore exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act.

Enclosed please find a copy of the GRAS notice, which includes a comprehensive summary of data supporting the safety of the ingredient and the signed statement of an expert panel regarding the value of these data in supporting a GRAS determination.

My contact information is provided below. Please feel free to contact me[1] by phone or e-mail if you have any questions regarding this GRAS notice.

Sincerely,

(b) (6)

(b) (6)

Michael Chen
Vice President of Sales and Marketing
Phone: 347-583-2601
Email: michael.chen@zcht.cc

[1] Please note that Nascent Health Sciences, LLC has authorized Dr. David Bechtel (David.Bechtel@Intertek.com) from Intertek Scientific & Regulatory Consultancy, located at 100 Davidson Avenue, Suite 102, Somerset, NJ 08873, to engage in discussions about any issues related to the enclosed GRAS notice. Dr. Bechtel may be reached by e-mail (shown above), by telephone at (908) 429-9202, or by FAX at (908) 429-9260.

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GRAS EXEMPTION CLAIM

SUMMARY OF DATA SUPPORTING A DETERMINATION
THAT THE USE OF PYRROLOQUINOLINE QUINONE (PQQ) DISODIUM
SALT IN SELECT FOODS IS
GENERALLY RECOGNIZED AS SAFE (GRAS)

Submitted to:

Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety

By:

Nascent Health Sciences, LLC
PO Box 325
Allentown, New Jersey
08501

February 1, 2016

GRAS Exemption Claim

Nascent Health Sciences, LLC has determined that the use of pyrroloquinoline quinine (PQQ) disodium salt under specific conditions of use as an ingredient in multiple food categories entails a reasonable certainty of no harm and is generally recognized as safe (GRAS) based on scientific procedures. Consequently, it is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act.

Signature (b) (6) Date 01-24-2016

Michael Chen
Vice President of Sales and Marketing

Name and Address of Notifier

Nascent Health Sciences, LLC
PO Box 325
Allentown, NJ 08501

Contact Name: Michael Chen
Phone: 347-583-2601
E-mail: michael.chen@zcht.cc

GRAS Substance

The subject of this GRAS notice is PQQ disodium salt from Nascent Health Sciences.. The product is manufactured under current good manufacturing practices (cGMP) (21 CFR, part 110). Inspections and testing are performed at various points during the manufacturing process; every lot of PQQ disodium salt is tested for compliance with the established specifications (*e.g.*, identity, heavy metals, microbiological activity).

Intended Use and Projected Consumer Exposure

Nascent Health Sciences, LLC intends to market PQQ disodium salt for use in foods as specified in Table 1.

Table 1 Summary of proposed food uses and maximum use levels

Food Category	Beverage-Uses	PQQ Disodium Salt Level (mg/serving)	RACC* (g or mL)	Use-Levels (mg/g)
Beverages and Beverage Bases	Energy, Sport, and Isotonic Drinks	8	240	0.0333
	Non-Milk Based Meal Replacement Beverages	8	240	0.0333
	Water (Bottled, Enhanced, Fortified)	8	240	0.0333

NHANES = National Health and Nutrition Examination Survey; PQQ = pyrroloquinoline quinone; U.S. = United States.
* RACC = Reference Amounts Customarily Consumed per Eating Occasion (21 CFR §101.12 – U.S. FDA, 2015b).

The mean and 90th percentile intakes in the consuming U.S. population resulting from these proposed uses were estimated to be 26.5 mg/person/day (0.4 mg/kg body weight/day) and 61.4 mg/person/day (0.9 mg/kg body weight/day), respectively.

Basis for GRAS Determination

To make the GRAS determination, Nascent Health Sciences, LLC compiled information about the substance, specifications, manufacturing, proposed uses, and evidence of safety into a comprehensive dossier (GRAS Dossier); and sought the opinion of qualified experts (*i.e.,* expert panel) in determining whether there is consensus among their peers that the use of this substance as described entails a reasonable certainty of no harm and is generally recognized as safe based on the available scientific evidence.

All data and information that are the basis for this GRAS determination are available for FDA's review and copying at reasonable times at Intertek Scientific & Regulatory Consultancy, 100 Davidson Avenue, Suite 102, Somerset, NJ 08873, and will be sent to FDA upon request.

GRAS Expert Evaluation Pyrroloquinoline Quinone Disodium Salt in Select Foods

Background

The undersigned, an independent panel of recognized experts (hereinafter referred to as the Expert Panel), qualified by scientific training and relevant international experience to evaluate the safety of food and food ingredients, was commissioned by Nascent Health Sciences, LLC (hereafter Nascent) to evaluate the generally recognized as safe (GRAS) status of pyrroloquinoline quinone (PQQ) disodium salt in select foods. Specifically, PQQ disodium salt is proposed for use in the United States in foods such as energy, sport, and isotonic drinks; non-milk based meal replacement beverages; water (bottled, enhanced, fortified); PQQ disodium salt is intended to be used in these foods at a maximum level of 8 mg PQQ disodium salt/serving for its nutritive value in supporting metabolic processes, primarily through its activity as an antioxidant. PQQ is also intended for use in dietary supplements.

The Expert Panel, independently and collectively, critically evaluated information contained in a comprehensive package of scientific information and data from the literature and other sources compiled by Intertek Scientific & Regulatory Consultancy. In a conference call on March 18, 2015, the Expert Panel unanimously concluded that the intended uses of PQQ disodium salt, manufactured according to current Good Manufacturing Practices (cGMP) and meeting the food-grade specifications presented in the dossier, is Generally Recognized as Safe (GRAS) based on scientific procedures.

Nascent Health Sciences' PQQ disodium salt is synthesized from ethyl 6-amino-5-methoxy-1H-indole-2-carboxylate in a complex 3-step chemical reaction and is chemically identical to PQQ occurring naturally in various fruits, vegetables, and beverages The Panel reviewed batch analysis data that indicates that this manufacturing method results in a finished product that reproducibly meets product specification stability data demonstrating that the product is stable over its labeled shelf-life.

The Expert Panel considered product specific safety studies have been conducted and support the safety of Nascent Health Sciences' PQQ disodium salt. In genotoxicity evaluations, PQQ disodium salt was investigated for its potential to induce mutations in the bacterial reverse mutation assay, the sperm malformation assay, and in an *in vivo* micronucleus test. No mutagenicity or genotoxicity was observed in any of these tests. The acute oral LD_{50} of PQQ disodium salt was shown to be 3,690 to 5,010 mg/kg bw in rats. In a published, GLP-compliant 13-week oral, repeated dose subchronic study in Sprague-Dawley rats, no unscheduled deaths occurred, and there were no treatment-related changes in food consumption or body weight gain. No toxicologically significant effects on hematology, serum biochemistry, or histopathology were observed. Histopathologic changes observed do not appear to be dose-related; in fact, the incidence of lesions in the various control groups renders the results to be spurious. The no-observed-adverse-effect-level (NOAEL) of PQQ disodium salt was considered to be 400 mg/kg bw/day in rats (Liang *et al*., 2014). No teratogenic effects were seen following administration of PQQ disodium salt to pregnant rats on Days 7 through 16 of gestation. These studies are also supported by other preclinical and clinical studies with PQQ disodium salt. A second 90-day study by Nakano *et al.* (2014) reported a NOAEL of 100 mg/kg/day.

PQQ disodium salt is intended to be used in these foods at a maximum level of 8 mg PQQ disodium salt/serving. These proposed uses would result in mean and 90th percentile all-user intakes of 26.5 and 61.4 mg/person/day, or 0.4 and 0.9 mg/kg bw/day, respectively. The Panel noted that these exposures are greater than 100-fold lower than the NOAEL reported in the 90-day rat study.

Conclusion

We, the Expert Panel, have independently and collectively critically evaluated the information summarized above and unanimously conclude that there is reasonable certainty that no harm will result from the use and intended use levels of Nascent Health Sciences' PQQ disodium salt. Pyrroloquinoline quinone (PQQ) disodium salt is proposed for use due to its nutritive value in the United States (U.S.) in foods, such as energy, sport, and isotonic drinks; non-milk based meal replacement beverages; and water (bottled, enhanced, fortified). PQQ is also intended for use in dietary supplements. PQQ disodium salt is intended to be used in these foods at a maximum level of 8 mg PQQ disodium salt/serving. These proposed uses would result in mean and 90th percentile all-user intakes of 26.5 and 61.4 mg/person/day, or 0.4 and 0.9 mg/kg bw/day, respectively. These exposures are greater than 100-fold lower than the NOAEL reported in the 90-day rat study.

Therefore, we conclude that the intended use and use levels of PQQ disodium salt, manufactured according to current Good Manufacturing Practices (cGMP) and meeting the food-grade specifications presented in the dossier, is Generally Recognized as Safe (GRAS) based on scientific procedures.

(b) (6)

04/13/2015

Robert J. Nicolosi, Ph.D.
Professor Emeritus, Department of Clinical Laboratory
& Nutritional Sciences
University of Massachusetts Lowell, MA

Date

4/14/15

John A. Thomas, Ph.D.
Adjunct Professor, Department of Pharmacology & Toxicology
Indiana University School of Medicine Indianapolis, IN

Date

(b

4/15/15

David H. Bechtel, Ph.D., D.A.B.T.
Vice President
Intertek Scientific & Regulatory Consultancy, Bridgewater, NJ

Date

SUMMARY OF DATA SUPPORTING A DETERMINATION THAT THE USE OF PYRROLOQUINOLINE QUINONE (PQQ) DISODIUM SALT IN SELECT FOODS IS GENERALLY RECOGNIZED AS SAFE (GRAS)

- Final -

Submitted To: Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety

Prepared by: Nascent Health Sciences, LLC.
PO Box 325
Allentown, New Jersey
08501

February 1, 2016

SUMMARY OF DATA SUPPORTING A DETERMINATION THAT THE USE OF PYRROLOQUINOLINE QUINONE (PQQ) DISODIUM SALT IN SELECT FOODS IS GENERALLY RECOGNIZED AS SAFE (GRAS)

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SUMMARY OF DATA SUPPORTING A DETERMINATION THAT THE USE OF PYRROLOQUINOLINE QUINONE (PQQ) DISODIUM SALT IN SELECT FOODS IS GENERALLY RECOGNIZED AS SAFE (GRAS)

1.0 Introduction

Nascent Health Sciences LLC (Nascent hereafter) has undertaken an independent safety evaluation of pyrroloquinoline quinone (PQQ) disodium salt. The purpose of the evaluation was to ascertain whether the use of PQQ disodium salt in select food products for the general human population might be considered generally recognized as safe (GRAS) through scientific procedures. Specifically, PQQ disodium salt is proposed for use in the United States in foods such as energy, sport, and isotonic drinks; non-milk based meal replacement beverages; and water (bottled, enhanced, fortified). PQQ disodium salt is intended to be used for nutritive value in these foods at a maximum level of 8 mg PQQ disodium salt/serving. PQQ is also intended for use in dietary supplements.

In the present GRAS dossier, Nascent provides detailed information about the intended foods and use levels, manufacturing, specifications, and batch analyses of PQQ disodium salt. Nascent conducted searches of the published scientific literature through December 2014 for information related to its safety in several medical and toxicological databases, as well as various reference texts, and on-line sources, including:

- TOXLINE
- PUBMED
- The National Toxicology Program (NTP) Chemical Health & Safety Information
- GENETOX
- ChemID*plus*
- Developmental and Reproductive Toxicology/Environmental Teratology Information Center (DART/ETIC)
- Hazardous Substances Databank (HSDB)

Nascent used the information summarized herein to provide a basis for a determination that the use of PQQ disodium salt in select food categories entails a reasonable certainty of no harm and is generally recognized as safe (GRAS) by scientific procedures. Such use would therefore be exempt from the premarket approval requirements of the Federal Food, Drug and Cosmetic Act.

2.0 GRAS Substance Characterization

2.1 Description of the Ingredient

The ingredient, PQQ disodium salt, disassociates to PQQ. PQQ is reportedly ubiquitous in the soil, bacteria, and plants. It has reportedly been found in all plant foods analyzed to date, suggesting humans have a long history of exposure to PQQ. Kumazawa *et al*. (1995) detected nanogram quantities of PQQ in various fruits, vegetables, and beverages. PQQ was also found in human milk at concentrations between 140 and 180 ng/mL (Mitchell *et al.,* 1999). It has also been detected in human serum, urine, cerebrospinal fluid, and adrenal tissue (Paz *et al*., 1988; Killgore *et al.*, 1989).

Although studies examining to the absorption, distribution, metabolism, and excretion of PQQ are limited, the available data indicate oral PQQ is bioavailable, distributed in the plasma, and excreted, primarily in the urine (See Section 5.0 for additional information). Physiological roles include function as a classical water soluble vitamin/cofactor, roles in cell signaling, and as an antioxidant. PQQ serves as a coenzyme to several bacterial dehydrogenases. Similarly, a PQQ-dependent dehydrogenase enzyme was identified in mice. PQQ was also shown to function as a mammalian redox cofactor in lysine metabolism (Salisbruy, 1979; Kashara and Kato, 2003). Diets deficient in PQQ resulted in impaired growth, immunological defects, and decreased fertility in mice (Killgore *et al*., 1989; Steinberg *et al.,* 1994; Rucker, 2002). PQQ confers radioprotection, neuroprotection and cardioprotection in *in vitro* and *in vivo* models (Rucker *et al.,* 2009; Xiong *et al*., 2011; Gong *et al*., 2012).

2.2 Chemistry Data

Chemical Name: Pyrroloquinoline Quinone Disodium Salt

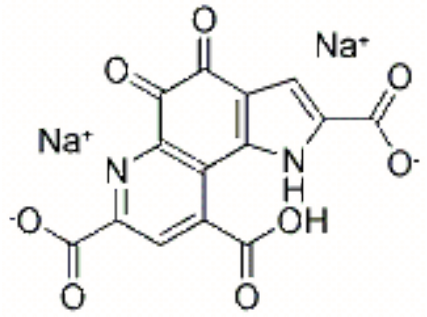
Synonyms: Methoxatin disodium salt, Disodium pyrroloquinolinedione tricarboxylate

Chemical Abstracts Service (CAS) Number: 122628-50-6

Molecular Formula: $C_{14}H_4N_2Na_2O_8$

Chemical Structure:

Figure 2-1 Structure of PQQ Disodium Salt



2.3 Manufacturing Process of New Dietary Ingredient

Nascent Health Sciences synthesizes PQQ disodium salt from ethyl 6-amino-5-methoxy-1H-indole-2-carboxylate *via* a 3-step chemical reaction. The manufacturing process, which complies with current Good Manufacturing Practice, is shown in Figure 2-2. Reaction byproducts are quantified in the final analysis of PQQ disodium salt using HPLC and are required to be less than 0.1% or not detectable. Figure 2-3 compares the HPLC chromatograms of Nascent's product to that of standard PQQ. The absence of additional peaks demonstrates byproducts are absent in Nascent's product.

The certificate of analysis and HPLC chromatogram for the starting material, ethyl 6-amino-5-methoxy-1H-indole-2-carboxylate, are included in Appendix 1. An HPLC chromatogram for PQQ is included in Appendix 2. The reagents involved in the synthesis of PQQ disodium salt from ethyl 6-amino-5-methoxy-1H-indole-2-carboxylate include dimethyl oxoglutaconate, copper acetate monohydrate, dichloromethane, sodium hydrogen carbonate, diethyl ether, HCL gas, ceric ammonium nitrate, acetonitrite, sodium sulfate, toluene, ethyl acetate, sodium hydroxide, sodium chloride, silicon dioxide, and ethanol. Purchasing specifications and risk analysis of raw materials are included in Appendix 3.

Figure 2-2 PQQ Synthesis Reaction Sequence

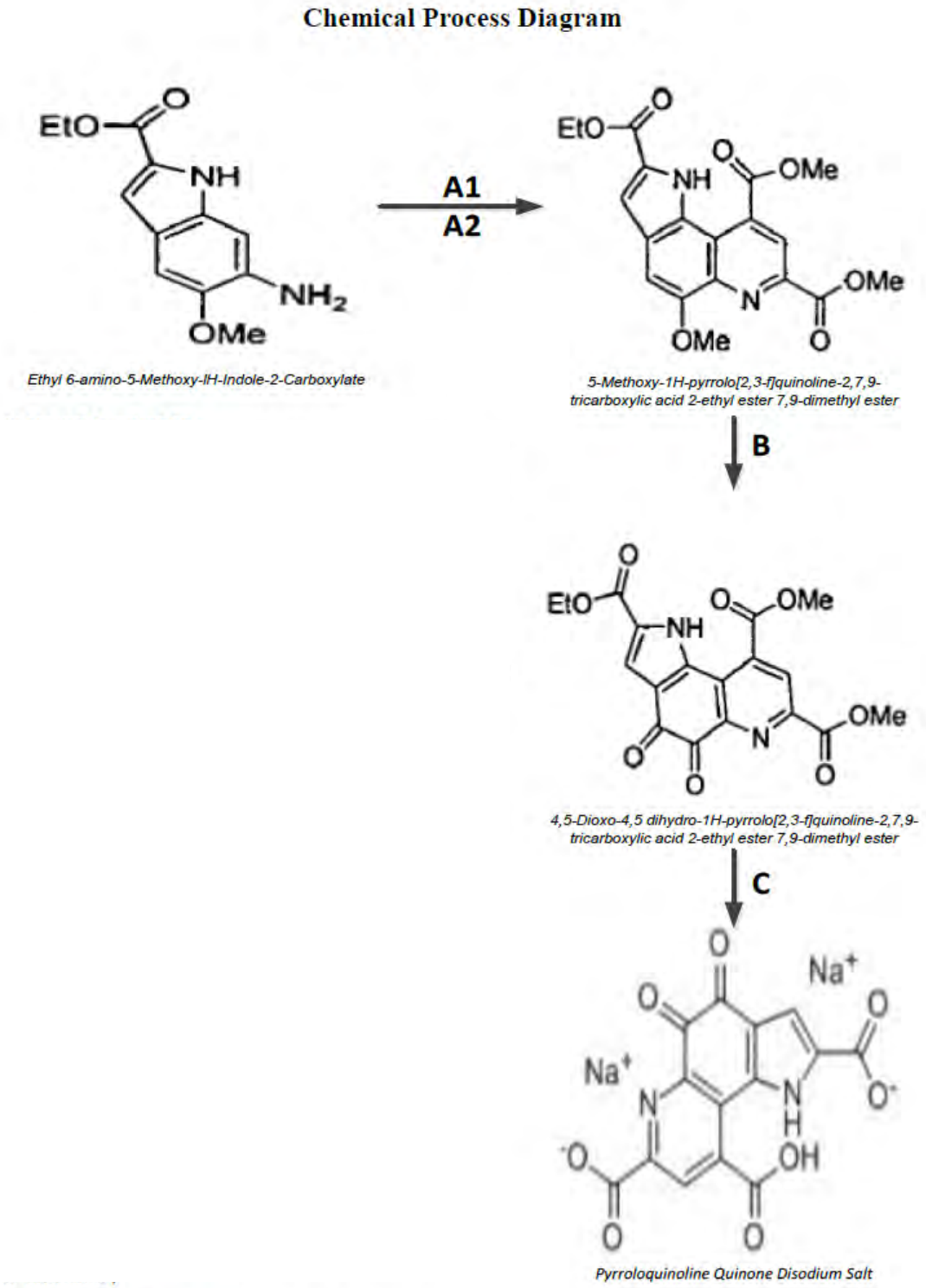


Figure 2-3 Manufacturing Flow Chart

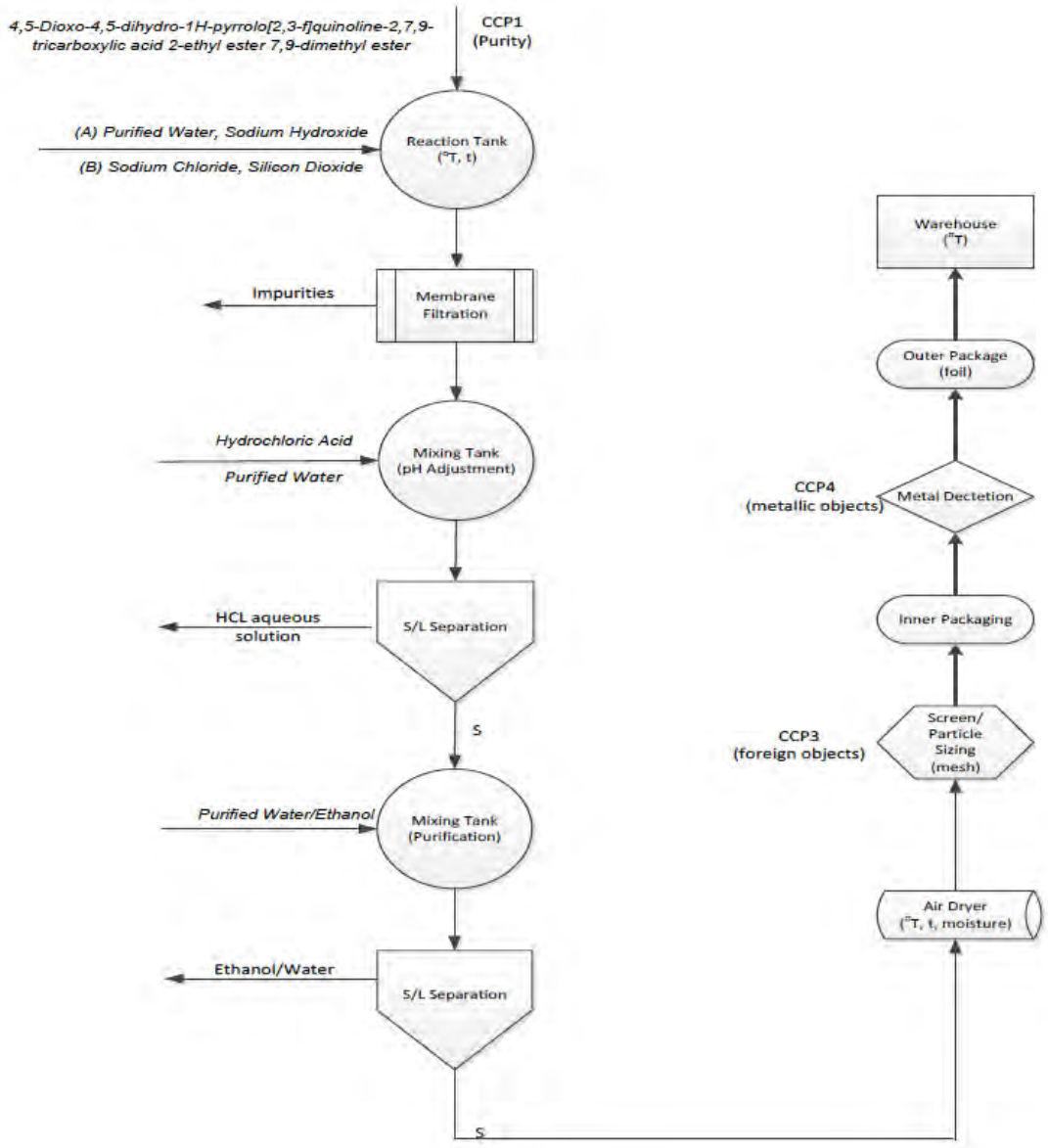
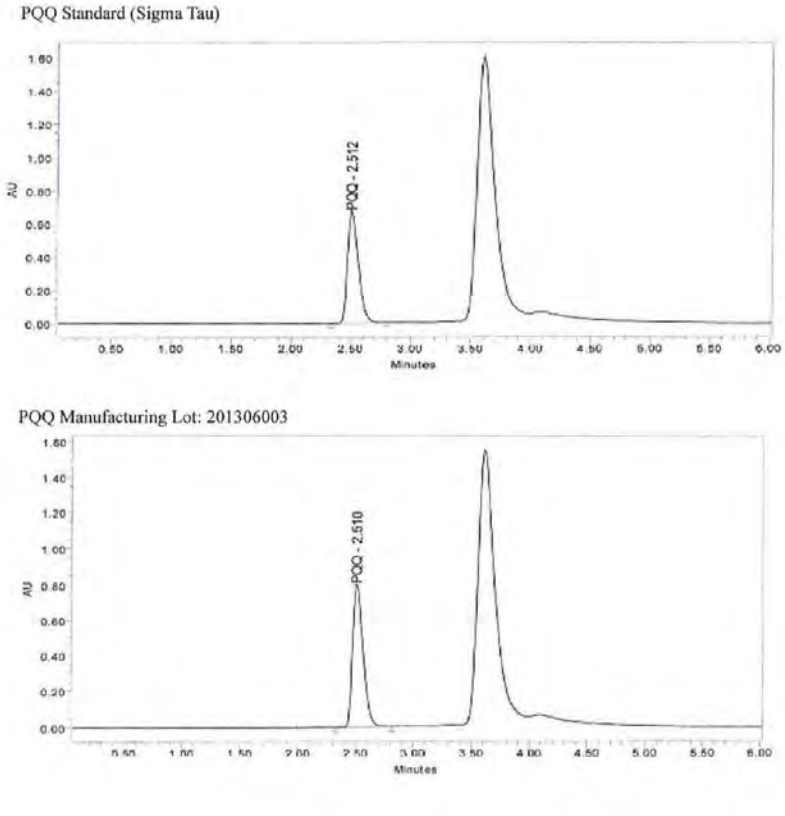


Figure 2-3 Comparative HPLC chromatogram of Nascent's PQQ to standard



2.4 Specifications and Batch Analyses Data for the Ingredient

The specifications for the new dietary ingredient, PQQ disodium salt, are summarized in Table 2-1. Batch analyses data for 6 non-consecutive batches is provided in Table 2-2. Results of a 6-month accelerated storage stability study are provided in Table 2-3. Product specifications and certificates of analysis are provided in Appendix 4.

Table 2-1 PQQ disodium salt product specifications and analytical methods (Nascent Health Sciences, 2015a)

SPECIFICATION	METHOD	PARAMETERS
SENSORY REQUIREMENTS		
Appearance	Visual	Crystalline Powder
Color	Visual	Reddish Brown
Taste	Taste	Salty
PHYSICAL-CHEMICAL REQUIREMENTS		
Identification	FTIR	Match Standard
Assay (dry basis)	HPLC[1]	Not less than 98 %
Loss on drying	USP<731>	Not more than 12 %
Particle size	USP<786>	Not less than 99 % through 20 mesh
Ash*	USP<281>	Not more than 1.0 %
CONTAMINANTS/ADDITIVES		
Heavy metals, as Pb	USP<231>	Not more than 10 ppm
Arsenic (As)	AAS	Not more than 1.0 ppm
Cadmium (Cd)	AAS	Not more than 1.0 ppm
Lead (Pb)	AAS	Not more than 0.5 ppm
Mercury (Hg)	AAS	Not more than 0.1 ppm
Residual solvent (ethanol)	USP<467>	Not more than 0.5 %
MICROBIOLOGICAL REQUIREMENTS*		
Aerobic Plate Count	USP<61>	Not more than 1000 cfu/g
Yeast & Mold	USP<61>	Not more than 100 cfu/g
Escherichia coli	USP<61>	Negative
Salmonella typhimurium	USP<61>	Negative/10G
PACKAGING, STORAGE & TRANSPORTATION		
Packaging	1 KG or 5 KG in a sealed foil bag, impervious to light and humidity with a sealed inner bag.	
Storage	Untampered package can be stored under room conditions; refrigeration preferred.	
Shelf Life	2 Years	
Country of Origin	China	

*Test could be performed only on a periodic basis.

Specifications for cerium and other residual solvents used in the manufacturing process were (*i.e.,* methylene chloride, ethyl acetate, toluene, and acetonitrile) were not established due to analysis indicating non-detectable levels (See Appendix 4)

[1] A copy of the HPLC method used for assessment of purity is provided in Appendix 5.

Table 2-2 Batch analyses data for PQQ disodium salt

SPECIFICATION	PARAMETERS	Batch # 20110413*	Batch # 201301011*	Batch # 201306003	Batch # 201310001	Batch # 201310002	Batch # 201310003
SENSORY REQUIREMENTS							
Appearance	Crystalline Powder	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms
Color	Reddish Brown	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms
Taste	Salty	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms
PHYSICAL-CHEMICAL REQUIREMENTS							
Identification	Match Standard	Positive	Positive	Positive	Positive	Positive	Positive
Assay (dry basis)	Not less than 98 %	99.0 %	99.3 %	99.5 %	99.1 %	99.1 %	99.1 %
Loss on drying	Not more than 12 %	8.7 %	5.0 %	3.4 %	4.8 %	4.6 %	5.0 %
Particle size	Not less than 99 % through 20 mesh	-	-	99.5 %	99.8 %	99.5 %	99.5 %
Ash*	Not more than 1.0 %	-	-	0.05 %	0.05 %	0.05 %	0.05 %
CONTAMINANTS/ADDITIVES							
Heavy metals, as Pb	Not more than 10 ppm	< 10 ppm	< 10 ppm	< 10 ppm	< 10 ppm	< 10 ppm	< 10 ppm
Arsenic (As)	Not more than 1.0 ppm	-	-	Not Detected	Not Detected	Not Detected	Not Detected
Cadmium (Cd)	Not more than 1.0 ppm	-	-	0.03 ppm	0.02 ppm	0.01 ppm	0.01 ppm
Lead (Pb)	Not more than 0.5 ppm	-	-	Not Detected	Not Detected	Not Detected	Not Detected
Mercury (Hg)	Not more than 0.1 ppm	-	-	Not Detected	Not Detected	Not Detected	Not Detected
Residual solvent (ethanol)	Not more than 0.5 %	0.1 %	0.13 %	0.12 %	0.1 %	0.2 %	0.1 %
MICROBIOLOGICAL REQUIREMENTS*							
Aerobic Plate Count	Not more than 1000 cfu/g	-	-	< 10 cfu/g	80 cfu/g	< 10 cfu/g	< 10 cfu/g
Yeast & Mold	Not more than 100 cfu/g	-	-	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g
E. coli	Negative	-	-	Negative	Negative	Negative	Negative
Salmonella	Negative/10G	-	-	Negative	Negative	Negative	Negative

*****Limited tests were performed for product specifications in the beginning. Over time new parameters were added for batches made after June 1. 2013

Table 2-3 PQQ disodium salt product Stability Data (Nascent Health Sciences, 2015b)

Conditions: Temperature: 40 ± 2 °C, Humidity: 75 ± 5%
Packaging: Foil bag with inner polyethylene liner

ITEM	LIMITS	STARTING VALUE	MONTH			
			1	2	3	6
Lot Number 0040130100						
Appearance	Red to Reddish Brown	Reddish Brown	Reddish Brown	Reddish Brown	Reddish Brown	Reddish Brown
Moisture	≤ 12.0%	2.3%	2.5 %	2.8%	2.7%	3.0%
Assay	≥ 98.0%	99.7%	99.4%	99.5%	99.1%	99.3%
Lot Number 0040131002						
Appearance	Red to Reddish Brown	Reddish Brown	Reddish Brown	Reddish Brown	Reddish Brown	Reddish Brown
Moisture	≤ 12.0%	2.5%	3.0%	2.8%	3.5%	3.4%
Assay	≥ 98.0%	99.1%	98.8%	99.0%	98.6%	98.5%
Lot Number 00401301003						
Appearance	Red to Reddish Brown	Reddish Brown	Reddish Brown	Reddish Brown	Reddish Brown	Reddish Brown
Moisture	≤ 12.0%	3.9%	4.0%	4.5%	4.3%	4.5%
Assay	≥ 98.0%	99.7%	99.5%	99.2%	99.3%	99.2%

3.0 Requirements for a Generally Recognized as Safe (GRAS) Determination

Nascent is interested in self-affirming through scientific procedures that the use of PQQ disodium salt in select food categories (see Section 4.0) is GRAS for its intended uses, in accordance with Volume 62, Number 74 of the April 17, 1997 Federal Register, Pages 18937 to 18964 (Proposed Rules, 21 CFR Part 170, *et al*.). Such GRAS uses would therefore be exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act.

General recognition of safety, *i.e*., a determination that there is consensus among qualified experts that the use of a substance in specific food applications entails a reasonable certainty of no harm and is Generally Recognized as Safe (GRAS), may be through: (1) experience based on common use in foods if the substance was used in foods prior to January 1, 1958, when the Food Additives Amendment was enacted; or (2) scientific procedures. The proposed GRAS rule indicates that "*…general recognition of safety through scientific procedures is based upon generally available and accepted scientific data, information, methods, or principles, which ordinarily are published. Thus, studies would be one of several types of scientific data and information that could support the technical element of a scientific procedures GRAS determination. However, depending on the circumstances, other scientific information such as that relating to chemical identity or characteristic properties of a substance, determination of substantial equivalence to products already approved as safe, as well as methods of manufacture, could support, and in some cases be sufficient to satisfy that element.*"

There are multiple elements required for GRAS determination by scientific procedures, including extent of exposure/consumption and cumulative effect of the substance in the diet. This report addresses these elements and provides a comprehensive discussion of safety, as specified in the proposed sections of 170.36 outlined below:

(c) Notifiers shall submit the following information:

(4) A detailed summary of the basis for the notifier's determination that a particular use of the notified substance is exempt from the premarket approval requirement of the act because such use is GRAS. Such determination may be based either on scientific procedures or on common use in food.

(i) For a GRAS determination through scientific procedures, such summary shall include:

(A) A comprehensive discussion of, and citations to, generally available and accepted scientific data, information, methods, or principles that the notifier relies on to establish safety, including a consideration of the probable consumption of the substance and the probable consumption of any substance formed in or on food because of its use and the

cumulative effects of the substance in the diet, taking into account any chemically or pharmacologically related substances in such diet;

(B) A comprehensive discussion of any reports of investigations or other information that may appear to be inconsistent with the GRAS determination; and

(C) The basis for concluding, in light of the data and information described under paragraphs (c)(1), (c)(2), (c)(3), (c)(4)(i)(A) and (c)(4)(i)(B) of this section, that there is consensus among experts qualified by scientific training and experience to evaluate the safety of the substances added to food that there is reasonable certainty that the substance is not harmful under the intended conditions of use.

4.0 Intended Food Uses and Projected Dietary Exposure

4.1 Intended Food Uses

PQQ has been reported to function as a water soluble vitamin/cofactor and as an antioxidant. Pyrroloquinoline quinone (PQQ) disodium salt is proposed for use in the United States (U.S.) in beverages such as energy, sport, or isotonic drinks, non-milk based meal replacement beverages, and bottled, enhanced, or fortified waters; PQQ disodium salt is also intended for use in dietary supplements.

Estimates for the intake of PQQ disodium salt were based on the proposed food-uses and use-levels for PQQ disodium salt in conjunction with food consumption data included in the U.S. National Center for Health Statistics' (NCHS) National Health and Nutrition Examination Surveys (NHANES) 2011-2012 (CDC, 2014; USDA, 2014) (See Appendix 6 for full report) Calculations for the mean and 90th percentile all-person and all-user intakes were performed for each of the individual proposed food-uses of PQQ disodium salt and the percentage of consumers were determined. Similar calculations were used to estimate the total intake of PQQ disodium salt resulting from all proposed food-uses of PQQ disodium salt combined. In both cases, the per person and per kilogram body weight intakes were reported for the following population groups:

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

The individual proposed food-uses and use-levels for PQQ disodium salt employed in the current intake analysis are summarized in Table 4-1. Food codes representative of each proposed food-use were chosen from the NHANES 2011-2012 (CDC, 2014; USDA, 2014). Food codes were grouped in food-use categories according to Title 21, Section §170.3 of the Code of Federal Regulations (CFR, 2014a). Product-specific adjustment factors were developed based on data provided in the standard recipe file for the Continuing Survey of Food Intakes by Individuals (CSFII) 1994-1996, 1998 survey (USDA, 2000).

[2] PQQ disodium salt is not intended for use in infant formula and infant foods and products containing it will not be marketed directly to this consumer group.

Table 4-1 Summary of the Individual Proposed Food-Uses and Use-Levels for PQQ disodium salt in the U.S. (2011-2012 NHANES Data)

Food Category	Food-Uses	PQQ Level (mg/serving)	RACC* (g or mL)	Use-Levels (%)
Beverages and Beverage Bases	Energy, Sport, and Isotonic Drinks	8	240	0.0333
	Non-Milk Based Meal Replacement Beverages	8	240	0.0333
	Water (Bottled, Enhanced, Fortified)	8	240	0.0333

NHANES = National Health and Nutrition Examination Survey; PQQ = pyrroloquinoline quinone; U.S. = United States.
* RACC = Reference Amounts Customarily Consumed per Eating Occasion (21 CFR §101.12 – U.S. FDA, 2015b).

4.2 Estimated Daily Intake of PQQ Disodium Salt from All Proposed Food-Uses in the U.S.

Table 4-2 summarizes the estimated total intake of PQQ (mg/person/day) from all proposed beverage-uses in the U.S. population group. Table 4.1-2 presents this data on a per kilogram body weight basis (mg/kg body weight/day). The percentage of users was greater than 33.4% of the population groups, with female teens having the greatest percentage of users at 65.8% (Table 4.1-1). Low user percentages within a population group typically lead to dissimilar results for the all-person and all-user consumption estimates; therefore, only the all-user intakes will be discussed in further detail as they represent the exposures expected for the consumer (target) population.

Among consumers in the total population, the mean and 90th percentile all-user intakes of PQQ were determined to be 26.5 and 61.4 mg/person/day, respectively. Of the individual population groups, male adults were determined to have the greatest mean and 90th percentile all-user intakes of PQQ on an absolute basis, at 32.7 and 69.7 mg/person/day, respectively, while infants and young children had the lowest mean and 90th percentile all-user intakes of 8.4 and 20.8 mg/person/day, respectively (Table 4-2).

Table 4-2 Summary of the Estimated Daily Intake of PQQ Disodium Salt from Proposed Food-Uses in the U.S. by Population Group (2011-2012 NHANES Data)

Population Group	Age Group (Years)	All-Person Consumption (mg/day)		All-Users Consumption (mg/day)			
		Mean	90th Percentile	% Users	n	Mean	90th Percentile
Infants	0 to 2	2.8	9.3	33.4	254	8.4	20.8
Children	3 to 11	6.1	18.9	49.7	858	12.2	27.0
Female Teenagers	12 to 19	13.7	35.6	65.8	334	20.8	42.9
Male Teenagers	12 to 19	14.8	41.5	60.1	314	24.7	58.3
Female Adults	20 and up	14.3	43.8	50.6	1,209	28.2	63.1
Male Adults	20 and up	15.4	52.2	47.0	1,091	32.7	69.7
Total Population	All Ages	13.2	41.6	50.0	4,060	26.5	61.4

NHANES = National Health and Nutrition Examination Survey; PQQ = pyrroloquinoline quinone; U.S. = United States.

On a body weight basis, the mean and 90th percentile all-person intakes in the total population were determined to be 0.2 and 0.6 mg/kg body weight/day, respectively. Among consumers, the mean and 90th percentile all-user intakes for all age groups were 0.4 and 0.9 mg/kg body weight/day, respectively. Infants and young children were identified as having the highest mean and 90th percentile all-user intakes of any population group, of 0.7 and 1.5 mg/kg body weight/day, respectively. However, it is noted that infants and young children do not represent the target consumer population for products containing PQQ and this is thus an overestimation of actual intakes expected in this age category. Female teens had the lowest mean and 90th percentile all-user intakes of 0.3 and 0.7 mg/kg body weight/day, respectively (Table 4-3).

Table 4-3 Summary of the Estimated Daily Per Kilogram Body Weight Intake of PQQ Disodium Salt from Proposed Food-Uses in the U.S. by Population Group (2011-2012 NHANES Data)

Population Group	Age Group (Years)	All-Person Consumption (mg/kg bw/day)		All-Users Consumption (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants	0 to 2	0.2	0.8	33.4	253	0.7	1.5
Children	3 to 11	0.2	0.7	49.7	858	0.4	1.0
Female Teenagers	12 to 19	0.2	0.6	66.0	326	0.3	0.7
Male Teenagers	12 to 19	0.2	0.6	60.1	313	0.4	0.8
Female Adults	20 and up	0.2	0.6	50.5	1,194	0.4	0.8
Male Adults	20 and up	0.2	0.6	46.9	1,079	0.4	0.9
Total Population	All Ages	0.2	0.6	49.8	4,023	0.4	0.9

bw = body weight; NHANES = National Health and Nutrition Examination Survey; PQQ = pyrroloquinoline quinone; U.S. = United States.

4.3 Estimated Daily Intake of PQQ Disodium Salt from Individual Proposed Food-Uses in the U.S.

The total U.S. population was identified as being significant consumers of bottled, enhanced, and fortified water (31.1 to 60.8% users) and energy, sport, and isotonic drinks (3.7 to 24.5% users). In terms of contribution to total mean intake of PQQ disodium salt, bottled, enhanced, and fortified water contributed 79.1 to 93.7% to total mean intakes, whereas non-milk based meal replacements contributed to less than 3.3% across all age categories (see Tables A-1 to A-7 and/or B-1 to B-7 for further details).

4.4 Summary of Consumption Data

Consumption data and information pertaining to the individual proposed beverage-uses of PQQ disodium salt were used to estimate the all-person and all-user intakes of PQQ disodium salt for specific demographic groups and for the total U.S. population. This type of intake methodology is generally considered to be "worst case" as a result of several conservative assumptions made in the consumption estimates. For example, it is often assumed that all food products within a food category contain the ingredient at the maximum specified level of use. In addition, it is well-established that the length of a dietary survey affects the estimated consumption of individual users. Short-term surveys, such as the typical 2- or 3-day dietary surveys, may overestimate the consumption of food products that are consumed relatively infrequently.

In summary, on an all-user basis, the mean and 90th percentile intakes in the consuming U.S. population were estimated to be 26.5 mg/person/day (0.4 mg/kg body weight/day) and 61.4 mg/person/day (0.9 mg/kg body weight/day), respectively. On a body weight basis, the mean and 90th percentile intake of PQQ disodium salt among teenagers and adults were 0.3 to 0.4 mg/kg body weight/day and 0.7 to 0.9 mg/kg body weight/day, respectively. Infants and young children (aged 2 years and under), who are not representative of the target population, had the highest mean and 90th percentile intake on a per body weight basis at 0.7 and 1.5 mg/kg body weight/day, respectively.

5.0 Absorption, Distribution, Metabolism, and Excretion

Ten male Swiss-Webster mice (age not specified) received an oral dose of 28 µg of isotopic radiolabeled PQQ dissolved in saline buffered with 5 mM potassium phosphate by gavage. Animals were maintained on a chemically defined amino acid-based diet and housed in metabolic cages. Only 3.3% of the administered dose was absorbed by the 6 hour time-point, while 62% of this dose was absorbed in the lower intestine after 24 hours. Eighty one percent of the absorbed dose excreted in the urine. The liver retained only a small percentage of the

absorbed PQQ (*i.e.,* 5.4% after 6 hr and 1.5% after 24 hr). Low concentrations were detected in the liver, suggesting that biliary elimination is not a major excretion route in mice. In the blood, nearly all of the PQQ (95 to 97%) was associated with the blood cell fraction at both 6 and 24 hours. At 6 hr, the blood cell fraction constituted about 10% of the absorbed label, which was diminished to 1.2% at 24 hr. An additional 1.3% of the administered dose was detected in the skin 24 hours after dosing. (Smidt *et al.,* 1991).

It should also be noted that non-qualitative methods used this study used for quantitation of PQQ so the radioactivity could have been from PQQ, its metabolites, or a combination thereof. The observation that only 3% of the administered radioactivity was absorbed at the 6 hour time-point suggests that PQQ may be poorly absorbed. Further evidence of poor absorption of PQQ is available in animal feeding studies, where dark/green colored cecal contents and feces were detected (*e.g.*, Liang *et al.*, 2014; Nakano *et al.*, 2014; see Sections 6.2.2 and 6.3.1). Species differences (pharmacokinetic data from mice, 90-day studies were in rats) may contribute to this discrepancy. Limited data from humans seems to suggest metabolism would be more in line with what was seen in rats. Following repeated consumption of PQQ disodium at graduating doses up to 0.3 mg/kg body weight/day (corresponding to 21 mg PQQ/day for a 70 kg individual) for a period of 21 days, only 0.1% of the administered PQQ dose was identified in the urine in the non-derivatized (free) form (Harris *et al.*, 2013).

6.0 Safety

6.1 Overview

The overall safety of PQQ disodium salt is supported by the results of product-specific toxicological studies (see Section 6.2). The toxicity of PQQ disodium salt has been investigated in an acute oral toxicity test, 90-day feeding study, and a teratogenicity study.[3] Additionally, genotoxicity/mutagenicity studies including, the Ames test, a sperm malformation assay, and bone marrow micronucleus study were performed. All toxicity studies were performed with PQQ disodium salt, batch number PQQ20110413. Nascent Health Sciences' studies followed GB15193 (Chinese National Standards), which are consistent with OECD Guidelines. Additional supporting data on the safety of other PQQ products have also been reported in the published literature and are summarized in Sections 6.3 and 6.4.

6.2 Preclinical Safety Studies on Nascent Health Sciences' PQQ Disodium Salt

6.2.1 Acute Toxicity Study

An acute toxicity study was conducted using the Horn's method according to the Chinese Toxicology Assessment Procedures and Methods for Food Safety (Chinese Standard GB15193.3-2003). Sprague-Dawley rats (5 rats/sex/group) received 1000, 2150, 4640, 10,000, or 21,500 mg/kg body weight (bw) of PQQ disodium salt by gavage and were observed for 14 days. The two highest doses (*i.e.*, 10,000 and 21,000 mg/kg bw) were administered as divided doses. Based on the results of this study, the LD_{50} was considered to be 5010 mg/kg bw (3440 to 7300 mg/kg/bw) in females and 3690 mg/kg bw (2710 to 5010 mg/kg/bw) in males. According to acute toxicity classification, PQQ disodium salt demonstrates low toxicity in rats (China Center for Disease Control and Prevention, National Center for Food Safety Risk Assessment, 2012a). No other information was provided.

6.2.2 GLP-Compliant Repeated-Dose Toxicity, 90 days feeding study

Weanling Sprague-Dawley rats, specific pathogen-free grade (SPF grade), were individually housed in suspended stainless steel, open-mesh cages in a light/dark cycle, temperature, and humidity controlled room. Eighty healthy rats (10/sex/group) received 0, 100, 200, and 400 mg/kg bw of PQQ disodium salt daily by gavage for 90 days. Distilled water was administered to the control group. Food and water were provided *ad libitum*. The highest dose (400 mg/kg bw) was about 10% of the LD_{50} established by the acute toxicity study. Clinical observations were conducted twice daily. Food consumption was measured once a week. Hematological parameters and serum chemistry were evaluated on the 46th and 91st day. At the end of the study, all rats were sacrificed and full necropsy was performed.

No mortality or adverse effects were observed. No significant difference was observed on the body weights, food intake, weight gain, food utilization rate, white blood cells (WBCs), red blood cells (RBCs), hemoglobin (HGB), platelet count (PLT), monocytes (MO) , alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine (CRE), cholesterol (CHO), triglycerides (TG), total protein (TP), lymphocytes (LYM) and albumin (ALB) of treated rats compared to control rats. Body weight and food intake data are presented in Figures 6-1 through 6-4. Results for hematology, biochemistry, and relative organ weights have been summarized in Tables 6-1 through 6-3. A statistically significant increase was observed in granulocytes of female rats receiving 200 mg/kg body weight of PQQ on the 46th day of the study. A statistically significant decrease was observed in the blood glucose of male rats receiving 100 mg/kg body weight of PQQ disodium salt on the 45th day of the study. Both observations were transient, not dose-dependent, were gender specific and were within the normal range; thus, these findings were considered to be of no biological significance. As shown in Table 6-4, lesions were noted in the heart, liver, kidney, and testes of both treated and control rats. The incidence and/or severity of these lesions was not treatment-related and therefore not

considered to be of indicative of toxic effects of PQQ disodium salt. Histopathological examination revealed no differences in the spleen, thymus, stomach, or adrenal glands. The observed histopathologic changes do not appear to be dose-related, and in fact, the incidence of lesions in the various control groups renders the results to be spurious. No significant effects were seen on organ weights or organ to body weight ratios of treated mice relative to control mice. No other signs of toxicity were observed. The no-observed-adverse-effect-level (NOAEL) of PQQ disodium salt was considered to be 400 mg/kg bw/day in rats, which is in excess of 100-fold the serving amount (Liang *et al*., 2014).

Figure 6-1 Body weights of male and female rats receiving PQQ disodium salt (Liang *et al*., 2014)

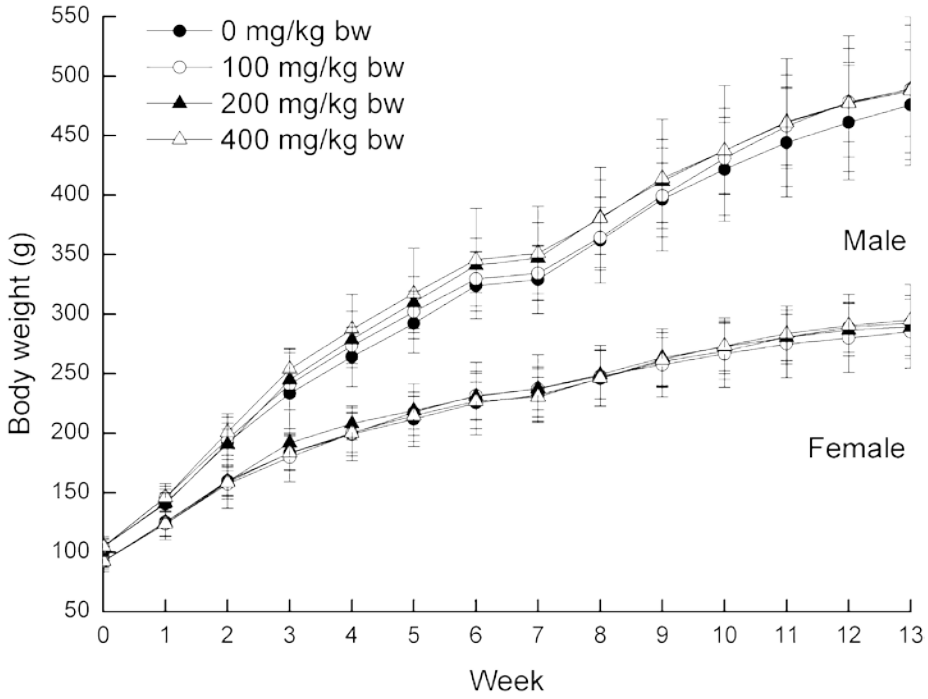


Figure 6-2 Food consumption among male and female rats receiving PQQ disodium salt (Liang *et al*., 2014)

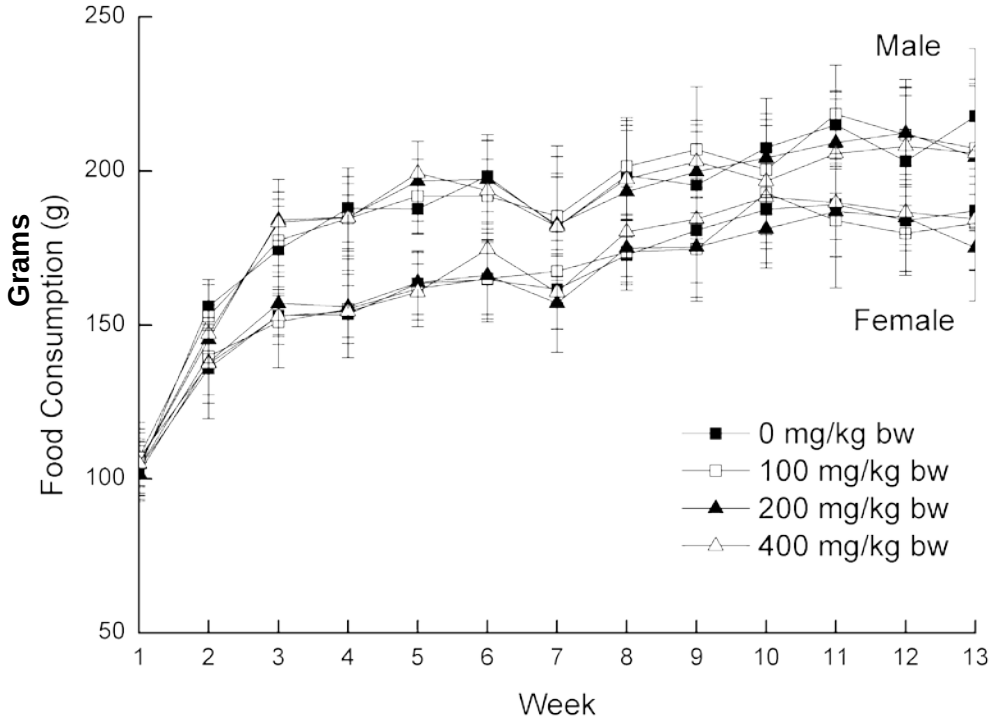


Table 6-1 Effects of PQQ disodium salt on rat hematology (Liang *et al.,* 2014)

Gender	Dosage (mg/kg bw)	Rats	Day	WBC ($x10^9$/L)	RBC ($x10^{12}$/L)	HBC (g/L)	PLT ($x10^{12}$/L)	LYM (%)	GR (%)	MO (%)
Female	0	10	46	6.57 ± 1.79	6.97 ± 0.46	151.3 ± 8.4	681.8 ± 116.1	74.4 ± 3.2	16.1 ± 2.8	9.5 ± 1.6
			91	7.26 ± 0.80	7.38 ± 0.31	167.6 ± 7.4	461.6 ± 65.8	73.9 ± 5.3	16.2 ± 4.6	9.9 ± 2.0
	100	10	46	7.30 ± 1.98	6.95 ± 0.39	154.2 ± 7.4	678.9 ± 138.9	73.4 ± 3.4	18.5 ± 2.9	8.1 ± 0.9
			91	8.17 ± 1.18	7.40 ± 0.30	160.3 ± 8.4	444.0 ± 69.4	76.3 ± 2.6	16.2 ± 2.4	7.5 ± 1.0
	200	10	46	8.68 ± 2.62	6.60 ± 0.27	144.8 ± 5.4	654.2 ± 81.9	71.2 ± 3.1	21.0 ± 2.4*	7.8 ± 1.7
			91	7.50 ± 2.14	7.19 ± 0.34	161.0 ± 9.6	467.3 ± 68.2	71.6 ± 4.6	18.5 ± 1.9	9.9 ± 4.4
	400	10	46	7.20 ± 1.72	7.14 ± 0.28	153.5 ± 8.6	622.8 ± 84.6	72.3 ± 6.7	18.9 ± 5.3	8.8 ± 2.4
			91	7.28 ± 1.40	7.26 ± 0.63	156.6 ± 12.6	408.5 ± 67.3	73.9 ± 3.5	17.7 ± 2.5	8.4 ± 2.1
Male	0	10	46	8.55 ± 2.05	7.12 ± 0.31	156.0 ± 5.7	846.6 ± 139.9	73.0 ± 2.05	18.6 ± 2.3	8.4 ± 1.7
			91	10.60 ± 0.95	7.33 ± 0.26	168.0 ± 17.5	656.8 ± 145.9	73.1 ± 1.4	19.6 ± 1.7	7.3 ± 1.2
	100	10	46	9.00 ± 2.00	7.09 ± 0.41	156.1 ± 4.5	793.8 ± 201.1	74.7 ± 4.4	16.0 ± 3.7	9.3 ± 2.6
			91	11.52 ± 2.75	7.41 ± 0.87	159.2 ± 13.4	597.0 ± 62.7	74.3 ± 2.7	17.4 ± 3.3	8.3 ± 2.1
	200	10	46	8.74 ± 1.90	7.24 ± 0.23	165.0 ± 11.7	853.2 ± 138.9	77.2 ± 4.2	15.5 ± 4.0	7.3 ± 1.7
			91	9.86 ± 1.95	7.52 ± 0.40	162.0 ± 13.0	625.3 ± 97.8	71.1 ± 4.2	20.3 ± 2.7	8.6 ± 2.1
	400	10	46	9.11 ± 2.47	7.24 ± 0.25	160.7 ± 6.7	730.1 ± 137.1	76.2 ± 3.0	16.2 ± 3.6	7.6 ± 1.5
			91	10.71 ± 2.88	7.42 ± 0.39	159.1 ± 6.2	553.3 ± 83.5	73.0 ± 4.0	18.7 ± 2.5	8.3 ± 2.1

Values represent mean ± SD.
WBC: white blood cells, RBC: red blood cells, PLT: platelet count, HBC: hemoglobin, LYM: lymphocytes, GR: granulocyte, MO: monocyte
* $P < 0.05$ as compared to the control.

Table 6-2 Effect of PQQ disodium salt on rat blood chemistry indices (Liang *et al.*, 2014)

Gender	Dosage (mg/kg bw)	No. of Rats	Day	ALT (U/L)	AST (U/L)	ALP (U/L)	BUN (mmol/L)	CRE (μmol/L)	CHO (mmol/L)	TG (mmol/L)	GLU (mmol/L)	TP (g/L)	ALB (g/L)
Female	0	10	46	35.4 ± 5.7	141.9 ± 18.2	100.2 ± 23.7	5.37 ± 1.61	61.9 ± 6.4	2.04 ± 0.42	0.44 ± 0.03	5.55 ± 0.59	68.0 ± 3.2	44.8 ± 1.7
			91	20.7 ± 4.3	147.2 ± 18.1	65.0 ± 18.0	5.25 ± 1.12	64.0 ± 3.1	1.96 ± 0.47	0.64 ± 0.16	4.44 ± 1.05	78.2 ± 4.2	44.9 ± 2.5
	100	10	46	40.7 ± 6.6	151.0 ± 23.6	119.5 ± 57.5	5.09 ± 1.32	62.4 ± 8.7	2.07 ± 0.43	0.49 ± 0.06	6.20 ± 0.72	69.6 ± 4.3	45.6 ± 2.2
			91	20.4 ± 8.2	157.6 ± 24.3	60.0 ± 18.0	5.19 ± 0.68	64.7 ± 7.5	2.07 ± 0.45	0.52 ± 0.05	4.94 ± 0.60	77.9 ± 3.7	45.1 ± 2.1
	200	10	46	42.5 ± 8.9	141.3 ± 20.1	124.2 ± 49.6	6.05 ± 1.68	62.9 ± 7.0	2.04 ± 0.45	0.56 ± 0.16	6.08 ± 0.94	69.3 ± 4.9	46.0 ± 3.0
			91	18.1 ± 5.7	159.7 ± 19.9	63.1 ± 13.8	5.46 ± 0.79	68.3 ± 3.4	1.82 ± 0.51	0.53 ± 0.13	4.91 ± 0.79	78.2 ± 3.0	45.1 ± 2.2
	400	10	46	37.4 ± 4.40	154.3 ± 22.4	90.1 ± 31.7	4.62 ± 0.80	59.8 ± 7.0	2.05 ± 0.52	0.54 ± 0.09	5.87 ± 1.00	70.5 ± 3.4	46.5 ± 1.4
			91	19.8 ± 3.2	156.7 ± 20.7	71.0 ± 24.6	5.57 ± 1.17	67.9 ± 5.5	2.11 ± 0.64	0.57 ± 0.13	4.62 ± 0.59	79.3 ± 1.8	45.9 ± 1.9
Male	0	10	46	39.3 ± 2.8	180.2 ± 19.3	154.2 ± 47.7	4.42 ± 0.94	58.1 ± 6.4	1.72 ± 0.38	0.51 ± 0.09	5.27 ± 0.41	67.2 ± 4.1	43.3 ± 2.1
			91	29.5 ± 9.6	171.4 ± 36.8	131.3 ± 22.6	5.88 ± 1.05	67.6 ± 5.9	1.89 ± 0.34	0.84 ± 0.30	4.39 ± 0.56	74.5 ± 3.3	41.7 ± 2.2
	100	10	46	38.8 ± 3.2	186.0 ± 30.1	138.0 ± 37.5	4.57 ± 1.03	57.1 ± 4.6	1.77 ± 0.38	0.59 ± 0.14	4.49 ± 0.31*	66.2 ± 4.2	43.6 ± 2.3
			91	28.2 ± 6.7	194.1 ± 32.6	134.2 ± 25.2	5.03 ± 0.62	62.9 ± 6.6	1.65 ± 0.25	0.77 ± 0.23	4.84 ± 0.53	71.9 ± 3.6	40.5 ± 1.5
	200	10	46	39.7 ± 4.1	184.9 ± 23.4	157.8 ± 51.8	4.19 ± 0.56	61.8 ± 5.2	2.00 ± 0.39	0.54 ± 0.09	5.27 ± 1.01	69.1 ± 5.3	44.8 ± 2.1
			91	24.6 ± 4.3	174.1 ± 18.8	117.9 ± 27.2	5.03 ± 0.61	66.1 ± 6.4	1.77 ± 0.35	0.59 ± 0.13	4.76 ± 0.53	75.8 ± 3.4	42.1 ± 1.0
	400	10	46	39.0 ± 3.9	191.2 ± 13.5	132.1 ± 36.4	4.94 ± 0.78	59.1 ± 5.9	1.74 ± 0.47	0.52 ± 0.09	5.00 ± 0.47	67.5 ± 5.1	44.4 ± 2.1
			91	27.8 ± 9.4	173.2 ± 17.5	123.8 ± 19.8	5.53 ± 1.16	68.5 ± 5.6	1.69 ± 0.38	0.66 ± 0.22	4.66 ± 0.46	74.2 ± 2.7	41.3 ± 0.9

Values represent mean ± SD.
*P < 0.05 as compared to the control.
ALT: alanine aminotransferase, ALP: alkaline phosphatase, AST: aspartate aminotransferase, BUN: blood urea nitrogen, CRE: creatinine, CHO: cholesterol, TG: Triglycerides, GLU: Glucose, TP: Total Protein, ALB: Albumin, GLU: glucose

Table 6-3 Organ-to-body weight ratio of rats receiving PQQ disodium salt for 13 weeks (Liang *et al.*, 2014)

Sex	Dose (g/kg bw)	No. of Rats	BW (g)	Liver		Spleen		Kidney		Thymus		Heart		Testicle	
				W (g)	Organ to BW (%)	W (g)	Organ to BW (%)	W (g)	Organ to BW (%)	W (g)	Organ to BW (%)	W (g)	Organ to BW (%)	W (g)	Organ to BW (%)
Female	0	10	268.5 ± 17.2	80.1 ± 0.71	3.02 ± 0.22	0.55 ± 0.07	0.21 ± 0.03	2.17 ± 0.18	0.82 ± 006	0.29 ± 0.09	0.11 ± 0.04	1.04 ± 0.12	0.39 ± 0.03	-	-
	0.1	10	260.6 ± 27.2	7.79 ± 0.89	3.00 ± 0.36	0.49 ± 0.07	0.19 ± 0.04	2.24 ± 0.22	0.86 ± 0.10	0.31 ± 0.10	0.12 ± 0.04	0.97 ± 0.07	0.37 ± 0.02	-	-
	0.2	10	263.9 ± 24.8	7.86 ± 1.17	2.97 ± 0.31	0.58 ± 0.14	0.22 ± 0.05	2.26 ± 0.20	0.86 ± 0.07	0.31 ± 0.11	0.12 ± 0.04	0.99 ± 0.11	0.38 ± 0.02	-	-
	0.4	10	269.2 ± 25.1	7.91 ± 1.31	2.94 ± 0.42	0.56 ± 0.11	0.21 ± 0.04	2.31 ± 0.16	0.86 ± 0.09	0.29 ± 0.08	0.11 ± 0.03	1.03 ± 0.14	0.38 ± 0.05	-	-
Male	0	10	446.5 ± 43.6	11.49 ± 1.16	2.61 ± 0.43	0.71 ± 0.16	0.16 ± 0.04	3.29 ± 0.42	0.75 ± 0.15	0.38 ± 0.05	0.08 ± 0.01	1.47 ± 0.13	0.33 ± 0.06	3.35 ± 0.18	0.76 ± 0.09
	0.1	10	452.6 ± 41.6	11.16 ± 1.40	2.46 ± 0.14	0.72 ± 0.10	0.16 ± 0.02	3.31 ± 0.34	0.73 ± 0.06	0.42 ± 0.10	0.09 ± 0.02	1.50 ± 0.19	0.33 ± 0.03	3.30 ± 0.33	0.73 ± 0.10
	0.2	10	454.5 ± 50.5	11.79 ± 1.65	2.60 ± 0.31	0.68 ± 0.10	0.15 ± 0.02	3.59 ± 0.44	0.80 ± 0.14	0.34 ± 0.07	0.07 ± 0.01	1.52 ± 0.15	0.34 ± 0.06	3.54 ± 0.31	0.78 ± 0.06
	0.4	10	452.7 ± 58.4	12.38 ± 1.66	2.74 ± 0.26	0.69 ± 0.12	0.15 ± 0.03	3.67 ± 0.74	0.82 ± 0.18	0.36 ± 0.07	0.08 ± 0.01	1.49 ± 0.20	0.33 ± 0.05	3.16 ± 0.29	0.70 ± 0.09

Values represent mean ± SD.
BW: body weight; W: weight

Table 6-4 Histopathological changes of rats treated orally with PQQ disodium salt for 13 weeks (Liang *et al.*, 2014)

Organs	Lesions	Dose[a]			
		0 mg/kg bw	100 mg/kg bw	200 mg/kg bw	400 mg/kg bw
Liver	Slight sporadic focal necrosis	0	0	2	0
	Spotty necrosis	3	1	3	1
Heart	Focal necrosis	3	1	2	5
Kidneys	Deposition of calcium salts in renal tubular	0	0	0	1
Testes	Testicular atrophy	0	0	0	1

[a]10 animals/sex/group

6.2.3 Genotoxicity Studies

The genotoxic potential of PQQ disodium salt was examined in 3 studies, namely an Ames bacterial mutagenicity assay, a sperm malformation assay, and an *in vivo* mouse bone marrow micronucleus study.

6.2.3.1 Ames Assay

The Ames assay, a reverse mutation assay, was used to investigate the mutagenic potential of PQQ disodium salt in *Salmonella typhimurium* strains TA97, TA98, TA100, and TA102. NaN_3 (sodium azide), 2-AF (2-aminofluorene), 4-nitro-O-Phenylenediamine, MMC (Mitomycin C), and 1,8-dihydroxyanthraquinone served as positive controls depending on the strain used and the presence or absence of metabolic activation. As shown in Table 6-5, concentrations of 0, 62, 556, 1667, and 5000 µg/plate were tested in all strains with and without metabolic activation. The experiment was run in duplicate. Positive control showed a significant increase in revertants, validating the assay. Data from the Ames assay demonstrates that PQQ disodium salt was non-mutagenic under the conditions of this study (China Center for Disease Control and Prevention, National Center for Food Safety Risk Assessment, 2012a). No other information was provided.

Table 6-5 Mean revertants per plate (with standard deviation)

		Dosage (µg/dish)	TA97		TA98		TA100		TA102	
			-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Test Material	Exp 1	62	138.3 ± 7.6	129.0 ± 24.6	41.0 ± 5.3	46.3 ± 2.3	121.0 ± 6.6	137.3 ± 20.0	291.3 ± 18.4	275.0 ± 1.0
	Exp 2		127.0 ± 11.5	132.3 ± 17.2	46.0 ± 10.6	49.0 ± 6.1	113.3 ± 3.8	146.0 ± 3.5	281.0 ± 5.2	282.7 ± 12.4
	Exp 1	185	134.0 ± 6.2	130.0 ± 21.0	42.3 ± 7.6	44.0 ± 9.6	147.3 ± 2.1	128.0 ± 16.5	293.0 ± 17.3	283.0 ± 27.1
	Exp 2		131.0 ± 25.1	140.0 ± 6.0	43.0 ± 8.7	48.7 ± 4.2	136.3 ± 18.6	129.0 ± 20.5	283.0 ± 23.6	284.7 ± 23.7
	Exp 1	556	139.3 ± 15.0	137.0 ± 20.3	52.7 ± 2.1	48.3 ± 10.7	120.0 ± 8.9	137.7 ± 19.2	296.0 ± 21.0	299.0 ± 18.0
	Exp 2		147.1 ± 22.7	120.3 ± 6.8	51.3 ± 3.1	45.3 ± 9.9	116.3 ± 8.6	142.3 ± 18.7	289.3 ± 25.8	278.7 ± 15.3
	Exp 1	1667	137.7 ± 7.4	131.3 ± 8.3	44.3 ± 2.9	46.0 ± 4.6	124.3 ± 17.2	151.3 ± 15.3	269.0 ± 7.8	298.0 ± 28.6
	Exp 2		142.3 ± 20.5	128.3 ± 14.2	37.0 ± 3.0	51.0 ± 1.0	125.3 ± 9.1	133.3 ± 15.6	271.7 ± 14.0	296.7 ± 32.6
	Exp 1	5000	134.7 ± 14.5	141.3 ± 19.7	40.3 ± 9.2	51.0 ± 6.9	145.3 ± 32.7	165.3 ± 11.7	272.0 ± 19.5	298.3 ± 6.1
	Exp 2		137.0 ± 22.1	137.7 ± 18.9	48.7 ± 5.8	44.3 ± 7.5	141.3 ± 9.0	148.0 ± 26.7	276.7 ± 9.3	294.3 ± 12.7
Control Group	Exp 1		144.0 ± 13.2	124.3 ± 13.1	48.0 ± 8.5	47.0 ± 3.5	132.3 ± 12.9	129.3 ± 15.4	291.0 ± 10.1	301.7 ± 17.0
	Exp 2		140.3 ± 18.6	142.0 ± 13.1	51.7 ± 5.7	47.7 ± 3.2	129.3 ± 4.2	141.3 ± 16.4	278.3 ± 7.6	287.7 ± 11.0
Solvent Control	Exp 1		147.0 ± 15.9	137.0 ±13.5	36.0 ± 2.6	36.0 ± 1.0	140.3 ± 7.6	146.3 ± 7.2	291.3 ± 25.6	269.0 ± 14.7
	Exp 2		140.3 ± 20.8	139.7 ± 16.2	43.0 ± 2.6	39.0 ± 5.3	142.3 ± 14.8	132.3 ± 17.2	287.0 ± 17.7	284.0 ± 24.6
Positive Control(µg/dish)										
NaN_3	Exp 1	1.5					1380.7 ± 224.0			
	Exp 2						1579.3 ± 119.0			
2-AF	Exp 1	10.0		1187.3 ± 140.0		2955.3 ± 83.7		1669.3 ± 209.8		
	Exp 2			1312.0 ± 149.0		2967.3 ± 213.8		1536.0 ± 228.1		
4-nitro-O-Phenylenediamine	Exp 1	20.0	1308.0 ± 256.8		1871.3 ± 193.4					
	Exp 2		1065.3 ± 205.2		1817.3 ± 151.5					
MMC	Exp 1	2.5							1845.3 ± 144.3	
	Exp 2								1638.7 ± 57.5	
1,8-dihydroxyanthraquinone	Exp 1	50.0								673.3 ± 56.8
	Exp 2									670.0 ± 84.9

6.2.3.2 Sperm Malformation Assay

The potential of PQQ disodium salt to increase the incidence of sperm malformation was evaluated in mice. Thirty-five Kunming mice were divided into five groups and exposed to 20 mL/kg body weight of PQQ disodium salt by oral gavage. Doses of 460, 920, and 1840 mg/kg body weight were administered daily for 5 days. The highest dose was established based on the acute toxicity study in mice. Distilled water and 40 mg/kg body weight of cyclophosphamide were administered as the negative and positive controls, respectively. There were no deaths reported in any of the mice following dosing. No clinical changes were reported in any of the control mice. Thirty days after the last PQQ disodium salt dose, 5 mice/group were randomly selected to be sacrificed. The epididymides were removed and examined microscopically and sperm aberration rate was calculated per 1000 sperm per mouse. Sperm were evaluated for sperm count and abnormal morphology such as amorphism, large-head, banana-shaped-head, double-heads, double-tail, and folded-tail. As shown in Table 6-6, no significant increase in sperm malformation in PQQ disodium salt-treated groups relative to the control group was observed. Positive control showed a significant increase in sperm malformation, validating the assay. Thus, it was concluded that PQQ disodium salt was non-mutagenic under the conditions of this assay (China Center for Disease Control and Prevention, National Center for Food Safety Risk Assessment, 2012a). No other information was provided.

Table 6-6 Effects of PQQ disodium salt on the incidence of mouse sperm malformation

Dosage (mg/kg bw)	Animal Number	Sperm Number	Sperm Malformation No.	Sperm Malformation Ratio
0	5	1000	21.0 ± 3.0	2.10 [a]
460	5	1000	21.8 ± 2.8	2.18
920	5	1000	23.2 ± 2.8	2.32
1840	5	1000	19.8 ± 3.1	1.98
Positive Control CP 40 mg/kg bw	5	1000	54.6 ± 3.5	5.46 [b]

[a] Comparing to each test material control, (X^2 test, P>0.05)
[b] Comparing to each test material control and negative control, (X^2 test, P<0.01)
CP: cyclophosphamide

6.2.3.3 Micronucleus Assay

In an *in vivo* mouse bone marrow micronucleus assay, five groups of Kunming mice (5/sex/group) were exposed to 20 mL/kg body weight of PQQ disodium salt twice by oral gavage in 24 hours. Doses of 630, 1250, and 2500 mg/kg body weight were administered to females and dose of 460, 920, and 1840 mg/kg body weight were administered to males. The highest doses were established based on the acute toxicity study in mice. Distilled water and 40 mg/kg body weight of cyclophosphamide were administered as the negative and positive controls, respectively. Mice were sacrificed 6 hours after the last administration. Bone marrow cells were collected from all mice and were examined microscopically for the presence of micronuclei. A summary of the results of the bone marrow and micronucleus assay of PQQ

disodium salt is presented in Table 6-7. There was no significant increase in the number of micronucleated PCEs in PQQ disodium salt-treated groups relative to vehicle control in either male or female mice at any dose level or bone marrow collection time. Positive control showed a significant increase in micronucleated polychromatic erythrocytes (PCEs), validating the assay. Thus, it was concluded, under the conditions of this study, PQQ disodium salt was non-genotoxic under the conditions of this assay (China Center for Disease Control and Prevention, National Center for Food Safety Risk Assessment, 2012a). No other information was provided.

Table 6-7 Incidence of polychromatic erythrocytes (PCEs) in mouse bone marrow following exposure to PQQ disodium salt

Gender	Dose (mg/kg bw)	No. of Mice	PCE			Micronucleus		
			RBC No.	PCE No.	PCE/RBC (%)	No. of observed PCE	No. of MN	Rate of MN (%)
Female	0	5	200	108.4 ± 4.2	54.2	1000	1.8 ± 1.3	1.8[a]
	630	5	200	108.0 ± 7.0	54.0	1000	1.6 ± 1.1	1.6
	1250	5	200	108.6 ± 6.9	54.3	1000	1.6 ± 0.9	1.6
	2500	5	200	108.4 ± 6.0	54.2	1000	2.0 ± 1.4	2.0
	CP 40 mg/kg bw	5	200	95.6 ± 6.8	47.8	1000	1.8 ± 3.8	18.0[b]
Male	0	5	200	109.6 ± 7.5	54.8	1000	1.6 ± 0.9	1.6[a]
	460	5	200	108.4 ± 5.1	54.2	1000	2.2 ± 1.3	2.2
	920	5	200	109.0 ± 4.1	54.5	1000	1.6 ± 0.5	1.6
	1840	5	200	108.8 ± 4.8	54.4	1000	1.2 ± 0.8	1.2
	CP 40 mg/kg bw	5	200	96.4 ± 5.6	48.2	1000	18.6 ± 2.9	18.6[b]

[a]: comparing to the test material dose group, (poisson distribution P>0.05)
[b]: comparing to the test material dose group and negative control (poisson distribution P<0.01)
PCE: polychromatic erythrocytes
MN: micronucleus
CP: cyclophosphamide

6.2.4 Teratogenicity Study

Pregnant Wistar rats (SPF grade) were randomly divided into 4 groups with at least 24 rats each. Rats were administered 0, 78, 310 and 1250 mg/kg body weight of PQQ disodium salt by oral gavage from the 7th to the 16th day of gestation. Doses were based on results from the acute toxicity study. Food and water were provided *ad libitum*. Pregnant rats were euthanized on the 20th day. Results are summarized in Tables 6-8, 6-9, and 6-10. 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 demonstrated significantly reduced body weights on days 9 and 12 compared to the rats receiving the negative control. However, because results were transient they were not considered to be toxicologically significant. No teratogenic effects were observed (China Center for Disease Control and Prevention, National Center for Food Safety Risk Assessment, 2012b). No other information was provided.

Table 6-8 PQQ disodium salt's influence on embryo survival and development

Effects of PQQ disodium salt on Embryonic Survival				
Dose (mg/kg bw)	**Live-Births/nest**	**Absorptive Fetuses/nest**	**Stillbirths/nest**	**Fetal Loss Rate (%)**
0	13.4 ± 2.6	0.7 ± 1.0	0	4.7
78	13.7 ± 4.0	0.7 ± 1.0	0	5.0
310	13.8 ± 3.9	0.8 ± 1.0	0	5.4
1250	14.9 ± 1.5	0.7 ± 1.1	0	3.8

Effects of PQQ disodium salt on Growth and Development of Fetal Rats (mean ± SD)			
Dosage (mg/kg bw)	**Observed fetal No. / nest**	**Weight (g)**	**Length (cm)**
0	13.4 ± 2.6	3.87 ± 0.43	4.16 ± 0.20
78	13.7 ± 4.0	4.05 ± 0.66	4.24 ± 0.25
310	13.8 ± 3.9	3.94 ± 0.56	4.15 ± 0.20
1250	14.9 ± 1.5	3.77 ± 0.43	4.17 ± 0.21

Table 6-9 Effects of PQQ disodium salt on skeletal development of fetal rat

Dose (mg/kg bw)	Observed Fetal Rat No./Nest	Skull Abnormalities/ Nest		Sternal Abnormalities/Nest		Other Skeletal Abnormalities/Nest		Skeletal Abnormality Rate (mg/kg bw)/ Nest (%)
		Malf. No.	Malf. Rate (%)	Malf. No.	Malf. Rate (%)	Malf. No.	Malf. Rate (%)	
0	6.9 ± 1.3	0.8 ± 1.0	10.6	1.3 ± 1.0	18.9	0.1 ± 0.4	1.3	19.6
78	7.0 ± 2.0	0.9 ± 1.4	12.7	1.0 ± 1.2	12.8	0 ± 0	0	21.1
310	7.2 ± 1.9	0.9 ± 0.9	11.6	1.2 ± 1.3	16.1	0 ± 0	0	19.1
1250	7.6 ± 0.8	0.8 ± 0.9	9.9	1.4 ± 1.0	18.1	0 ± 0	0	20.5

Malf. : Malformation

Table 6-10 Effects of PQQ disodium salt on the weight of pregnant rats (mean ± SD)

Dose (mg/kg bw)	Day 0 (g)	Day 3 (g)	Day 7 (g)	Day 9 (g)	Day 12 (g)	Day 16 (g)	Day 18 (g)	Day 20 (g)
0	267.2 ± 12.8	274.8 ± 13.6	284.7 ± 17.8	293.8 ± 17.9	309.8 ± 20.1	341.3 ± 28.9	368.4 ± 39.2	398.2 ± 49.8
78	267.9 ± 16.9	275.5 ± 19.9	284.0 ± 22.1	291.2 ± 23.9	309.4 ± 29.5	343.8 ± 36.4	372.0 ± 41.3	397.7 ± 47.7
310	267.5 ± 13.1	274.8 ± 15.3	284.0 ± 17.8	290.9 ± 16.2	307.7 ± 20.8	344.8 ± 35.1	371.0 ± 45.2	395.8 ± 56.3
1250	266.2 ± 10.7	272.1 ± 10.7	280.0 ± 13.5	283.2 ± 13.9*	296.2 ± 18.4*	328.3 ± 30.0	361.3 ± 41.9	389.4 ± 55.2

*compared with negative group $P < 0.05$

6.3 Preclinical Safety Studies on Other PQQ Products

6.3.1 BioPQQ™

BioPQQ™ (PQQ disodium salt from Mitsubishi Gas Chemical Co. Inc.) was evaluated in *in vitro* (Nakano *et al.,* 2013) and *in vivo* (Nakano *et al*., 2014) assays. These studies are summarized in Table 6-11 and 6-12, respectively.

Table 6-11 Genotoxicity assays for BioPQQ™

Study Type	Test System	S9	Exposure	Positive Control	Results
Ames test	*Salmonella typhimurium* strains: TA98, TA100, TA1535, TA1537 and *Echerichia coli* strain WP2*uvrA*	-S9	0, 10, 20, 39, 78, 156, 313, 625, 1250, 2500, 5000 µg/plate for 48 hrs	AF-2, NaN_3, ICR-191	Not mutagenic.
		+S9	0, 156, 313, 625, 1250, 2500, 5000 µg/plate for 48 hrs	B[a]P, 2AA	
Chromosome aberration	Lung fibroblast from new born, female Chinese hamsters	-S9	0, 12.5, 25, 50, 100, 200, 400 µg/mL for 6 and 24 hrs	MMC	Not mutagenic. [a]
		+S9	0, 117.2, 234.4, 468.8, 937.5, 1875, 3750 µg/mLfor 6 hrs	B[a]P	
Chromosome aberration	Human peripheral blood lymphocytes from non-smoking male and female adults	-S9	0, 234.4, 468.8, 937.5, 1875, 3750 µg/mL for 48 hrs AND 0, 117.2, 234.4, 468.8, 937.5, 1875, 3750 µg/mL for 24 hrs	MMS	Not mutagenic.
		+S9	0, 234.4, 468.8, 937.5, 1875, 3750 µg/mL for 48 hrs	cyclophosphamide	
In vivo micronucleus test	Crlj:CD1 (ICR) male mice	N/A	0, 250, 500, 1000, 2000 mg/kg bw	MMC	Not mutagenic.

[a] A statistically significant increase in the incidence of structural aberrations was observed in the first experiment after 6 hrs of exposure to 200 µg/mL in the absence of S9. Authors declared this "weakly" positive due to a low incidence of structural aberrations in the negative control based on historical data. Furthermore the incidence of structural aberrations observed was below 5%, indicating the result could not be classified as "positive."

Nakano *et al*., (2014) investigated the effects of BioPQQ™ in acute, 14-day dose-range finding, 28- day renal toxicity (with 4-week recovery period), and 13-week oral toxicity studies in Sprague-Dawley rats. BioPQQ™, suspended in 0.5% aqueous methylcellulose solution was administered by gavage. Acute, 14-day, and 13-week studies were conducted in accordance with GLP. Four (only acute) or five-week-old rats were purchased, quarantined, and acclimated for one week before initiation of studies. Animals were housed individually under controlled environmental conditions regulating 20.1-25.2 ºC temperature, 32.9-61.2% relative humidity, 13-17 fresh air exchanges/hour, and 12-hour light/dark cycles. Pelleted food (CRF-1) and tap water were available *ad libitum*.

The acute oral LD_{50} value for males was reported to range from 1000-2000 mg/kg/bw. The corresponding LD_{50} in females was 500-1000 mg/kg bw. In the 14-day study dose-ranging study, no adverse effects with respect to clinical observations, body weight, food consumption, hematology, blood biochemistry, or mortalities were observed in rats (6/sex/group) following

gavage administration of PQQ disodium salt at doses up to 768 mg/kg body weight/day. Although green-colored feces and cecal contents were observed in some rats in the groups given the 2 highest doses, feces were otherwise normal. Kidney weights were increased by 13% ($p<0.05$) relative to controls in females; however, this effect was not dose-responsive and no similar trend was observed in the males. Slight to moderate focal basophilic changes and atrophy of the renal tubules were reported in the high-dose female group. In males, minimal to slight basophilic atrophy also was observed in all treatment groups, including the control.

Based on the apparent renal findings observed in the acute and 14-day repeat-dose toxicity studies, a 28-day repeat-dose follow-up study in female SD rats (12/group) was conducted. The doses selected for this study (200 and 700 mg/kg bw/d) were based on the results of the 14-day dose-range study discussed above. 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 body weight 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). The apparent slight to moderate elevations in the incidences of urinary crystals and protein in female rats administered PQQ disodium salt were considered unlikely to be of toxicological significance since the findings were not dose-responsive, resolved following the recovery period, and were not associated with any biochemical or histopathological changes indicative of kidney toxicity.

In the 90-day study, no toxicologically significant effects were reported with respect to hematology, clinical biochemistry, urinalysis, gross necropsy, or histopathology when rats (10/sex/group) were administered 0, 3, 20, or 100 mg PQQ/kg body weight/day by oral gavage (Nakano *et al.,* 2014). Based on the results of these 3 studies, the no-observed-adverse-effect level (NOAEL) for PQQ was determined to be 100 mg/kg body weight/day.

Table 6-12 *In vivo* oral toxicity studies for BioPQQ™ (Nakano *et al*., 2014)

Study Type	Animal number (rats/group)	Exposure (mg/kg bw)	Endpoints Measured	Results
Acute	10/sex	Single dose of 0, 500, 1000, 2000	Clinical observations, body weight, and necropsy	LD_{50} for males: 1000-2000 mg/kg bw LD_{50} for females: 500-1000 mg/kg bw
14-day dose-range finding	6/sex	3, 12, 48, 92, 768 per day	Clinical observations, body weight, food consumption, hematology, clinical biochemistry, urinalysis, gross necropsy, histopathology	A significant increase in sodium levels at 768 mg/kg bw/day in males and females. Authors attributed the increase in sodium levels to the exposure to BioPQQ™. Increased relative kidney weights were paired with focal basophilic changes and atrophy of the renal tubules at 768 mg/kg bw/day in females. No other treatment related effects observed.
28-day renal toxicity	12 females*	0, 200, 700	Clinical observations, body weight, water consumption, clinical biochemistry, urinalysis, necropsy, histopathology	No deaths or significant adverse clinical findings noted in any treated groups throughout the study period. Although the presence of protein and crystals in the urine sediment at 200 mg/ kg bw/d and 700 mg/kg bw/d were considered treatment-related, 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 resolved during the four-week recovery period.
13-week	10/sex	0, 3, 20, 100	Clinical observations, body weight, food consumption, ophthalmology, clinical chemistry, hematology, urinalysis, gross necropsy, histopathology	No deaths occurred in any group throughout the study period. There were no significant clinical findings observed in any group during the study period, with the exception of green-colored feces in some male and female rats of the high-dose (100 mg/kg bw/d) group during days 7–11, and for all animals in this group during days 12–90, which were attributed to the fecal excretion of the dark-colored test substance. There were no significant differences in body weight gain or food consumption throughout the study period compared to the control groups and no ophthalmological abnormalities were noted in any animal. There were no significant differences in hematology or urinalysis parameters in any treatment group compared to the control group. Statistically increased AST activity was seen in the low-dose (3 mg/kg bw/d) group, decreased total cholesterol concentration was observed in the mid-dose (20 mg/kg bw/d) group, and decreased triglyceride concentration was reported in the high-dose group. These effects were considered physiological variations, as they were within the normal historical range for the laboratory, and were considered to be incidental due to the lack of dose dependency. No treatment-related effects were seen on histopathological examination. The NOAEL was considered to be 100 mg/kg bw/day, the highest dose tested..

*Six animals were euthanized at the end of the 28-day period; remaining six rats were monitored for a 28-day recovery period.

6.3.2 Additional PQQ Sources

Ten male Wistar rats (age not specified) were kept at 24 ± 2 °C and 55 ± 10% humidly under an alternating 12 hr light-dark cycle. Food was provided *ad libitum*. Five rats received PQQ dissolved in 2% (w/v) sodium biocarbonate solution intraperitoneally for 4 consecutive days at daily doses of 11.5 mg/kg body weight (10 mL/kg). Five control rats received the vehicle only. No significant differences were seen in the body weights of PQQ-treated rats relative to controls. Urinalysis revealed increased excretion of protein, glucose, ketone body, and occult blood, although the statistical significance of these changes was not addressed. Blood urea nitrogen and serum creatinine levels were significantly higher in PQQ-treated rats, while serum triglycerides were significantly lower. Serum glutamate pyruvate transaminase and glutamate oxaloacetate transaminase activities were also significantly higher in PQQ-treated rats. Gross examination revealed swelling of the kidneys, which was accompanied by increased absolute and relative kidney weights, the latter of which was significant. Evidence of renal tubular damage (*i.e*., vacuolar degeneration, atrophy, and necrosis of the proximal tubular epithelium in the renal cortex, dilation and regeneration of the tubules) was seen upon histopathological examination (Watanabe *et al*., 1989).

Zhu *et al*., 2006 compared the cardioprotective effectiveness of pyrroloquinoline quinone (PQQ) with metoprolol. The authors noted that high-dose PQQ (20 mg/kg) produced renal and hepatic toxicity, however, details were not provided. An intravenous dose of 15 mg/kg was not associated with renal or hepatic toxicity as reported by Zhu et al. (2004). Furthermore, it is important to note route differences between these studies and the proposed food uses for PQQ. In the Zhu *et al*. (2006) and Watanabe *et al.* (1989) papers, effects on the kidney were reported after i.v. administration of 20 mg/kg or i.p. administration of 11.5 mg/kg. In these cases, the kidney sees bolus dose, which would not occur following ingestion; the time and intensity of the challenge to the kidney is not comparable.

6.4 Clinical Studies

Clinical studies with PQQ are summarized in Table 5-12. Many of these studies were designed to evaluate the efficacy of PQQ and only included limited safety evaluations. No adverse effects were reported in these studies.

Table 6-13 Clinical studies with PQQ

Reference	Study Type	Subjects receiving test material	PQQ Exposure	Comments related to Safety
Harris *et al*., 2013	Preliminary clinical	10 (5/sex). Mean age: 28.1	0.075 mg/kg bw/day for 7 days 0.15 mg/kg bw/day for 7 days 0.3 mg/kg bw/day for 7 days	Approximately 1% of PQQ consumed was recovered in the urine as nonderivatized PQQ. Serum concentrations of nonderivatized PQQ increased significantly.
	Cross-over design	10 (5/sex). Mean age: 28.1	Single dose at 0.2 mg/kg bw	Authors indicate cholesterol, creatinine, glucose, LDL, HDL, TG, uric acid, total protein, and AAT isozyme activity were within normal range. Levels of PQQ peaked in serum in about 2 hours.
Harris *et al*., 2013 [a]	Cross-over design	10 (5/sex). Mean age: 28.1	0.3 mg/kg bw for 3 days	Authors indicate cholesterol, creatinine, glucose, LDL, HDL, TG, uric acid, total protein, and AAT isozyme activity were within normal range.
Rucker *et al*., 2012	Cross-over design	10	10-20 mg/day for 3 days	Authors indicated no changes in clinical indices.
Nakano *et al*., 2009	Double-blind, placebo-controlled, parallel group	22 healthy adults (9 male, 13 female)	20 mg/day for 12 weeks	Efficacy study that measured performance on mental function tests Authors indicated no adverse effects or abnormal findings related to the test material were observed, although it was not clear what endpoints were measured.
Nakano *et al*., 2012	Open-label	17 males and females	20 mg/day BioPQQ™ for 8 wks	No adverse effects related to BioPQQ™, as measured by subjective symptoms, objective findings and abnormal changes in the measured values (*e.g,.* weight, BMI, heart rate, blood pressure), were observed.
Unpublished data. **Cited in:** Nakano *et al*., 2014	Two prospective, randomized, parallel group, placebo-controlled, double-blind	Information not provided	60 mg/day BioPQQ™ for 4 wks	Authors indicate no adverse effects related to BioPQQ™ were observed, although it was not clear what endpoints were measured .

6.5 New Dietary Ingredient Notification for PQQ

In September of 2013, Nascent Health Sciences submitted a New Dietary Ingredient (NDI) notification to FDA. The agency responded with questions related to product chemistry and safety. The original notification reported the presence of hydropic degeneration in epithelial cells in the renal tubules in the 90-day study. In the absence of a complete histopathology report (because it was unclear to the agency how many animals had this lesion or how the lesion distribution and severity differed between the treated animals verses the control animals), it was not clear to the agency that this observation was inconsequential. As a result, the agency

concluded that Nascent's notification failed to provide an adequate basis to conclude that a dietary supplement containing PQQ, under the conditions recommended or suggested on the product label, would reasonably be expected to be safe. In March of 2014, Nascent submitted a revised NDI notification providing additional detail related to product chemistry and safety. The agency again failed to accept the notification, indicating that synthetic PQQ does not fall into any of the dietary ingredient categories within 21 U.S.C. 321(ff)(l). The agency also again expressed concern that the lack of a complete histopathology report detailing severity of the kidney changes and incidence in the various treatment groups was of concern.

Further communication provided by Xudong Jai (Director, Laboratory of Toxicology) at China National Center for Food Safety Risk Assessment where the study was conducted indicates that sporadic degeneration of renal tubule epithelial cells was observed in a few rats (2 animals for the control group and 2 animals for the high dose group). This change was not considered treatment-related because it was only sporadically detected and there is no significant difference between the control and treatment groups. The synthetic nature of Nascent's ingredient does not affect its eligibility for GRAS status.

6.5.1 Consumer Exposure to PQQ as a Dietary Supplement

Nascent Health Sciences manufactures PQQ in bulk powder and will supply to manufacturers for incorporation into finished dietary supplements. These supplements would be in the form of capsules, tablets, or other suitable dietary supplement forms. Any additional food ingredients and/or excipients those manufacturers might utilize to create blends or formulations including PQQ in supplements to be marketed to consumers will be specified in the New Dietary Ingredient submissions they will provide for these retail products as required under Section 413b of the act (21 U.S.C. 350b). Although Nascent Health Sciences recognizes that recommendations for frequency and dosing of use will be specified by dietary supplement manufacturers in the New Dietary Ingredient submissions they will provide for retail products including Nascent Health Sciences' PQQ under Section 413b of the act (21 U.S.C. 350b), Nascent Health Sciences recommends use with a dosage of 10-20 mg PQQ/day. Nascent Health Sciences does not intend for products containing its PQQ to be used by pregnant women or children.

7.0 Conclusion

Nascent Health Sciences' PQQ disodium salt is synthesized from ethyl 6-amino-5-methoxy-1H-indole-2-carboxylate in a complex 3-step chemical reaction. Batch analysis data indicate that this manufacturing method results in a finished product that reproducibly meets product specifications. Reaction byproducts are quantified in the final analysis of PQQ disodium salt using HPLC and are required to be below the limit of detection.

PQQ is reportedly ubiquitous in the soil, bacteria, and plants and has been detected in various fruits, vegetables, and beverages, including human milk. It has also been detected in human serum, urine, cerebrospinal fluid, and adrenal tissue. Although studies examining the absorption, distribution, metabolism, and excretion of PQQ are limited, the available data indicate oral PQQ is bioavailable, distributed in the plasma, and excreted, primarily in the urine.

Physiological roles include function as a classical water soluble vitamin/cofactor, roles in cell signaling, and as an antioxidant. Diets deficient in PQQ resulted in impaired growth, immunological defects, and decreased fertility in mice. PQQ reportedly confers radioprotection, neuroprotection and cardioprotection in *in vitro* and *in vivo* models.

PQQ disodium salt is proposed for nutritive use in energy, sport, and isotonic drinks; non-milk based meal replacement beverages; and water (bottled, enhanced, fortified). PQQ disodium salt is intended to be used in these foods at a maximum level of 8 mg PQQ disodium salt/serving. These proposed uses would result in mean and 90th percentile all-user intakes of 26.5 and 61.4 mg/person/day, or 0.4 and 0.9 mg/kg bw/day, respectively. These exposures are greater than 100-fold lower than the NOAEL of 100 mg/kg bw/day reported in the 90-day rat study of Nakano *et al.* (2014). PQQ is also intended for use in dietary supplements.

Product specific safety studies have been conducted and support the safety of Nascent Health Sciences' PQQ disodium salt. In genotoxicity evaluations, PQQ disodium salt was investigated for its potential to induce mutations in the bacterial reverse mutation assay, the sperm malformation assay, and in an *in vivo* micronucleus test. No mutagenicity or genotoxicity was observed in any of these tests. The acute oral LD_{50} of PQQ disodium salt was shown to be 3,690 to 5,010 mg/kg bw in rats. In a 13-week oral, repeated dose subchronic study in Sprague-Dawley rats, no unscheduled deaths occurred, and there were no treatment-related changes in food consumption or body weight gain. No toxicologically significant effects on hematology, serum biochemistry, or histopathology were observed. The observed histopathologic changes observed do not appear to be dose-related, and in fact, the incidence of lesions in the various control groups renders the results to be spurious. The no-observed-adverse-effect-level (NOAEL) of PQQ disodium salt was considered to be 400 mg/kg bw/day in rats (Liang *et al*., 2014). The NOAEL, 400 mg/kg, is in excess of 100 fold of the 90th percentile all-user intakes of PQQ resulting from the proposed uses of PQQ. No teratogenic effects were seen following

administration of PQQ disodium salt to pregnant rats on Days 7 through 16 of gestation.

These studies are supported by other preclinical and clinical studies with PQQ. PQQ was not genotoxic or mutagenic. Effects on the kidney were reported following intraperitoneal administration of PQQ. Similarly, Nakano *et al*. (2014) reported increased relative kidney weights were paired with focal basophilic changes and atrophy of the renal tubules at 768 mg/kg bw/day in females in a 14-day dose range finding study. These changes were found to be reversible in a follow-up 28-day study. Furthermore, no toxicologically significant effects were seen on the kidney in either Nascent's 13-week study or in the 13-week study of BioPQQ™. The NOAEL in the 90-day study by Nakano *et al.* (2014) was considered to be 100 mg/kg bw/day.

Information summarized herein provides a basis for a determination that there is consensus among qualified experts that the intended use of Nascent Health Sciences' PQQ disodium salt in select food categories entails a reasonable certainty of no harm and is generally recognized as safe (GRAS) by scientific procedures.

8.0 References

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APPENDIX 1

Certificate of Analysis and HPLC Chromatogram for Ethyl 6-amino-5-Methoxy-IH-Indole-2-Carboxylate

WEIFANG SHENGYU PHARMACEUTICAL CO., LTD

YANGKOZHEN HUANBOHAI INDUSTRIAL PARK, SHOUGUANG, SHANDONG, PRC
FAX NUMBER: 0536-5456368

CERTIFICATE OF ANALYSIS

Product Name	ETHYL 6-AMINO-5-METHOXY-1H-INDOLE-2-CARBOXYLATE		
Lot Number	(b) (6)	Batch Size	30 kg
Mfg. Date	2013.11.22	Expiration	2014.11.21
Testing Reference	Internal Specification		

ITEM	REQUIREMENT	RESULT
Appearance	Grey to light brown powder	greyish powder
Assay	Not less than 97.0%	97.5%
Moisture	Not more than 1.0%	0.37%
Melting Point	160-166°	162.8
Ash	Not more than 0.2%	0.11%
Total heavy metals	Not more than 10 PPM	<10 PPM

Conclusion: Meet specifications

WEIFANG SHENGYU PHARMACEUTICAL CO., LTD
YANGKOZHEN HUANBOHAI INDUSTRIAL PARK, SHOUGUANG, SHANDONG, PRC
FAX NUMBER: 0536-5456368

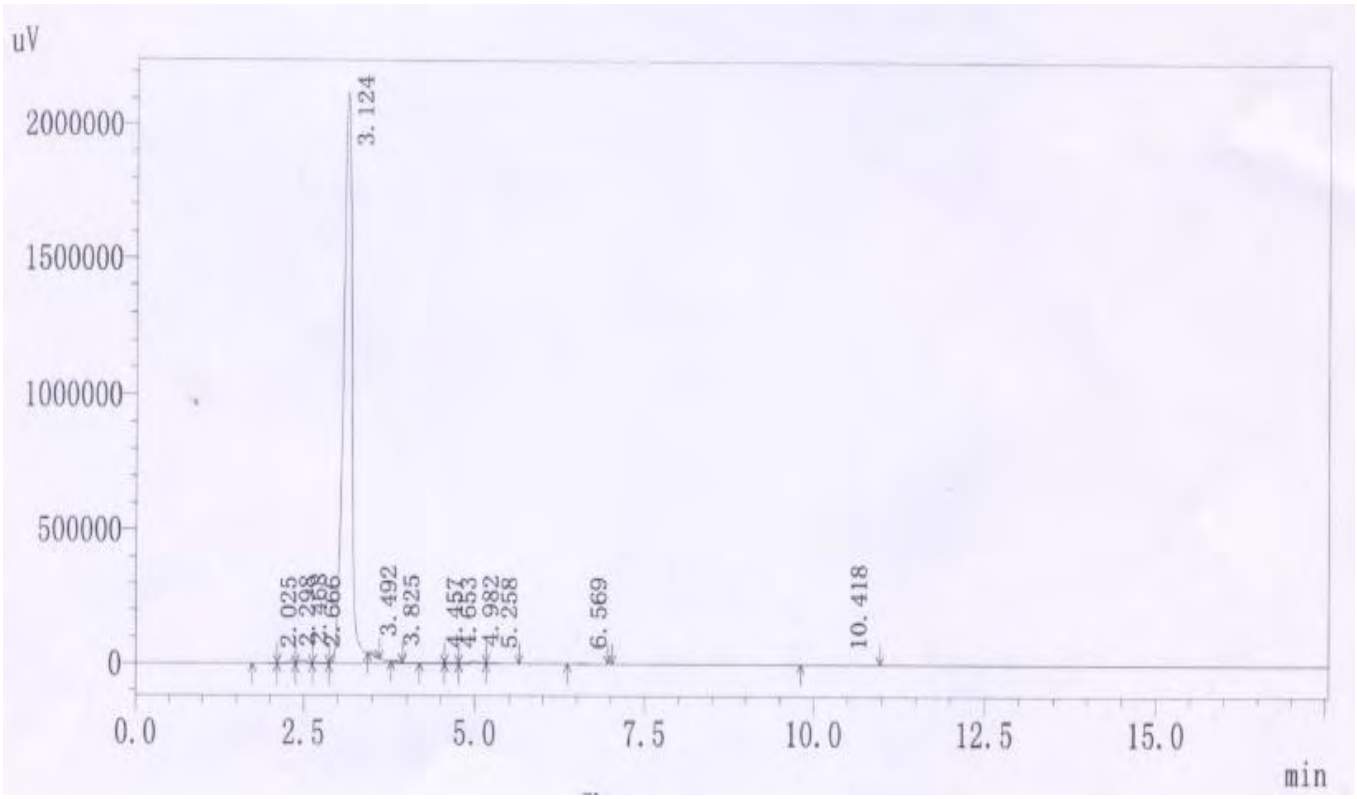
CHROMATOGRAM

ETHYL 6-AMINO-5-METHOXY-1H-INDOLE-2-CARBOXYLATE

Equipment No.: HPLC A

Data Gathered: 2013-11-23 11:03:05

Data Processed: 2013-11-23 11:21:28



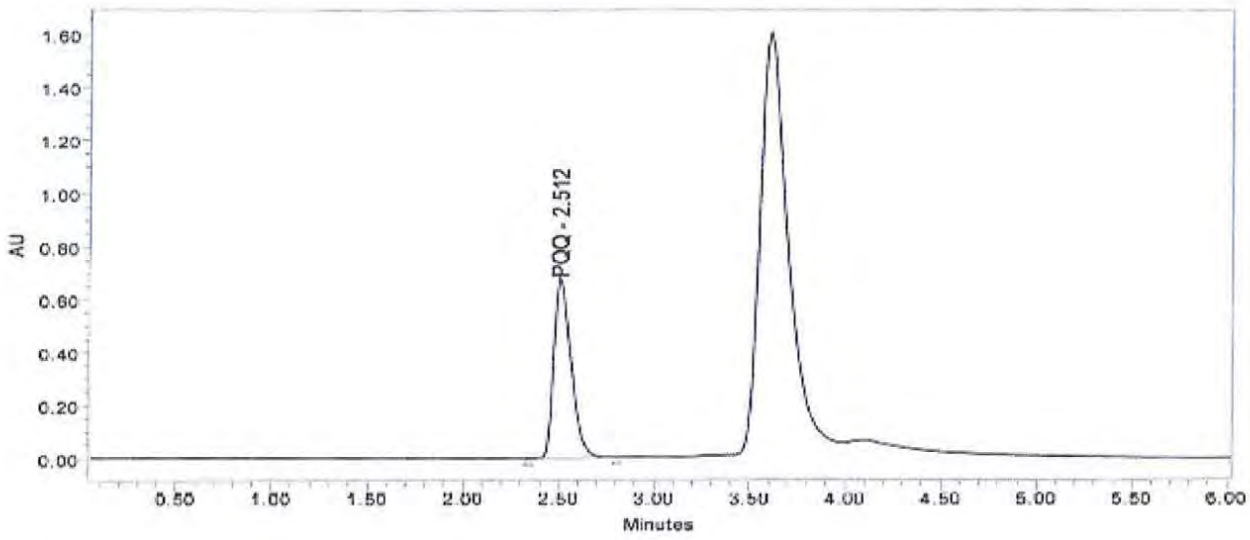
APPENDIX 2

HPLC Chromatogram for PQQ

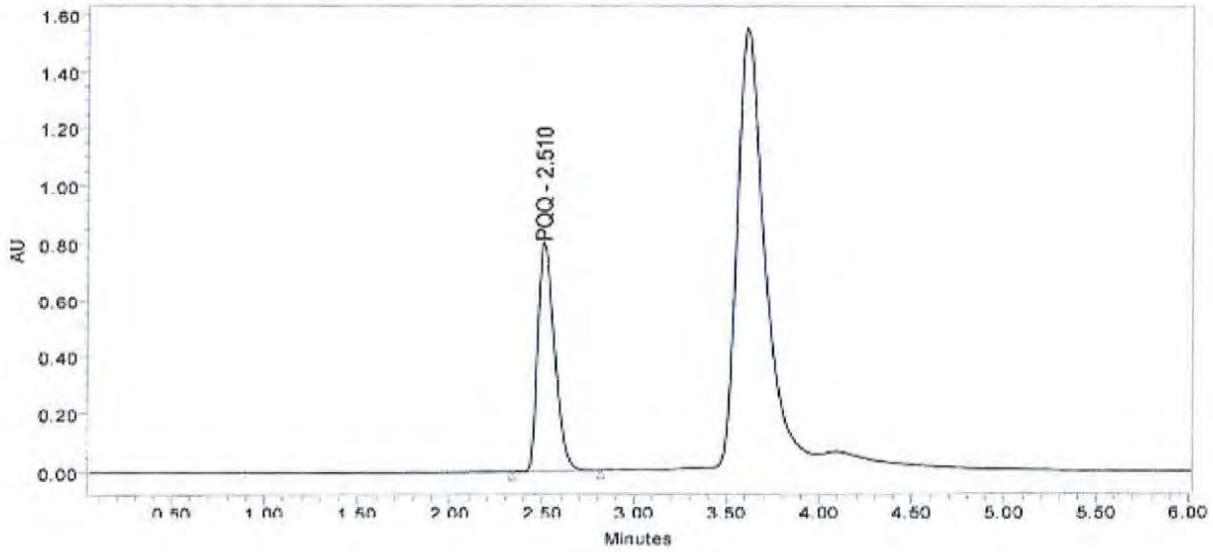
PYRROLOQUINOLINE QUINONE DISODIUM SALT

CHROMATOGRAM

PQQ Standard (Sigma Tau)



PQQ Manufacturing Lot: (b) (6)



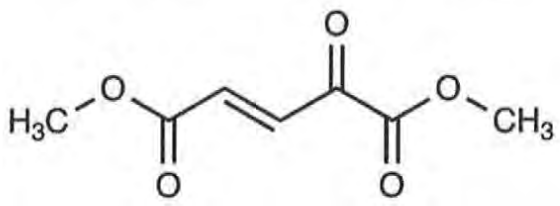
ZHUCHENG HAOTIAN PHARM CO..LTD
XINXING TOWN, ZHUCHENG, SHANDONG 262218 CHINA
TEL: 0086.536.6349756 FAX: 0086.536.6523666

APPENDIX 3

Purchasing Specifications and Risk Analysis of Raw Materials

ZCHT 浩天药业 HAOTIAN PHARMA

DIMETHYL OXOGLUTACONATE



GENERAL INFORMATION

CAS REGISTRY NUMBER:	78939-37-4
MOLECULAR FORMULA:	$C_7H_8O_5$
MOLECULAR WEIGHT:	172.14

CHEMICAL AND PHYSICAL REQUIREMENTS

APPEARANCE:	YELLOW POWDER
PURITY:	>95.0%
MELTING POINT:	52-54°C
ASH	<0.2%
TOTAL HEAVY METALS as Pb	<10 ppm

PACKAGING, SAFETY AND HANDLING

PACKAGING:	25KG POLYLINED BAG
STORAGE:	IN DRY SPACE UNDER REFRIGERATION
SHELF LIFE	1 YEAR
SAFETY:	IRRITATING TO EYES, RESPIRATORY AND SKIN; WEAR SUITABLE GLOVES AND GOGGLES IN CASE OF CONTACT, RINSE IMMEDIATELY WITH WATER AND SEEK MEDICAL ADVICE

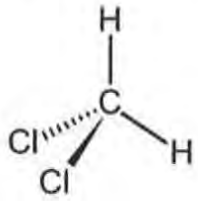
APPROVED SUPPLIER(S)

WEIFANG SHENGYU PHARMA COMPANY

ZHUCHENG HAOTIAN PHARM CO..LTD
XINXING TOWN, ZHUCHENG, SHANDONG 262218 CHINA
TEL: 0086.536.6349756 FAX: 0086.536.6523666

ZCHT 浩天药业 HAOTIAN PHARMA

DICHLOROMETHANE



GENERAL INFORMATION

CAS REGISTRY NUMBER:	75-09-2
MOLECULAR FORMULA:	CH_2Cl_2
MOLECULAR WEIGHT:	84.93

CHEMICAL AND PHYSICAL REQUIREMENTS

APPEARANCE:	COLORLESS LIQUID
PURITY:	>99.0%
BOILING POINT	39.6°C

PACKAGING, SAFETY AND HANDLING

PACKAGING:	10 GALLON DRUMS
STORAGE:	IN DRY SPACE AT AMBIENT CONDITIONS
SHELF LIFE	2 YEAR
SAFETY:	HARMFUL; FULL PROTECTIVE GOWNING IS NECESSARY WHEN HANDLING

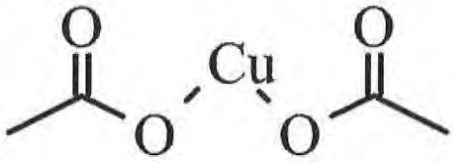
APPROVED SUPPLIER(S)

DONGYING SHIRONG CHEMICAL COMPANY

TIANJING BAISHI CHEMICAL COMPANY

ZCHT 浩天药业 HAOTIAN PHARMA

COPPER ACETATE MONOHYDRATE



GENERAL INFORMATION

CAS REGISTRY NUMBER:	6046-93-1
MOLECULAR FORMULA:	$Cu(OAc)_2 H_2O$
MOLECULAR WEIGHT:	199.65

CHEMICAL AND PHYSICAL REQUIREMENTS

APPEARANCE:	GREEN CRYSTALLINE POWDER
PURITY:	>99.0%
MELTING POINT:	115°C
ASH	<0.2%
TOTAL HEAVY METALS as Pb	<10 ppm

PACKAGING, SAFETY AND HANDLING

PACKAGING:	25KG POLYLINED BAG
STORAGE:	IN DRY SPACE AT AMBIENT CONDITIONS
SHELF LIFE	2 YEARS
SAFETY:	STRONG OXIDING AGENT; MAY CAUSE COMBUSTION AND FIRE WHEN IN CONTACT WITH OTHER SUBSTANCES. IT MAY CAUSE IRRITATION TO THE EYES, SKIN, AND THE RESPIRATORY TRACT.

APPROVED SUPPLIER(S)

SHIJIAZHUANG DITUO COPPER COMPANY
HANGZHOU SAGE CHEMICAL COMPANY

ZHUCHENG HAOTIAN PHARM CO..LTD
XINXING TOWN, ZHUCHENG, SHANDONG 262218 CHINA
TEL: 0086.536.6349756 FAX: 0086.536.6523666

ZCHT 浩天药业 HAOTIAN PHARMA

HYDROGEN CHLORIDE

GENERAL INFORMATION

CAS REGISTRY NUMBER:	7647-01-0
MOLECULAR FORMULA:	HCl
MOLECULAR WEIGHT:	36.46

CHEMICAL AND PHYSICAL REQUIREMENTS

APPEARANCE:	COLORLESS GAS
ODOUR	PUNGENT
PURITY:	>99.0
ACIDITY:	-7.0

PACKAGING, SAFETY AND HANDLING

PACKAGING:	HIGH-PRESSURIZED CYLINDER
STORAGE:	IN DRY SPACE AT AMBIENT CONDITIONS
SHELF LIFE	1 YEAR
SAFETY:	POISON GAS; CORROSIVE FULL PROTECTIVE GOWNING IS NECESSARY WHEN HANDLING

APPROVED SUPPLIER(S)

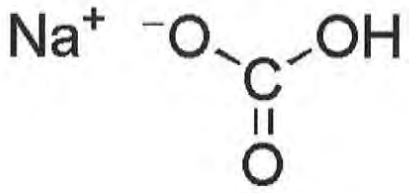
JINAN HAOHUA INDUSTRY CO., LTD
ZHEJIANG KAILI INDUSTRIAL CO., LTD

ZHUCHENG HAOTIAN PHARM CO..LTD
XINXING TOWN, ZHUCHENG, SHANDONG 262218 CHINA
TEL: 0086.536.6349756 FAX: 0086.536.6523666

PURCHASING SPECIFICATION - RAW MATERIAL

ZCHT 浩天药业 HAOTIAN PHARMA

SODIUM HYDROGEN CARBONATE



GENERAL INFORMATION

CAS REGISTRY NUMBER:	144-55-8
MOLECULAR FORMULA:	$NaHCO_3$
MOLECULAR WEIGHT:	84.01

CHEMICAL AND PHYSICAL REQUIREMENTS

APPEARANCE:	WHITE CRYSTALLINE POWDER
PURITY:	>99.0%
MELTING POINT:	50°C
ASH	0.2%
TOTAL HEAVY METALS as Pb	<10 ppm

PACKAGING, SAFETY AND HANDLING

PACKAGING:	25KG POLYLINED BAG
STORAGE:	IN DRY SPACE AT AMBIENT CONDITIONS
SHELF LIFE	2 YEAR
SAFETY:	MAY CAUSE EYE IRRITATION

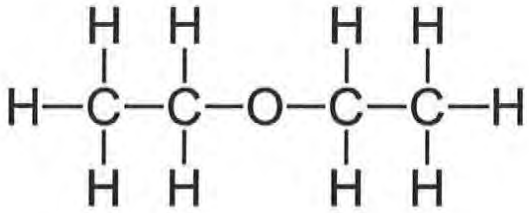
APPROVED SUPPLIER(S)

TIANJIN YUANLONG CHEMICAL COMPANY
JINZHOU CHANGSHENG CHEMICAL COMPANY

ZHUCHENG HAOTIAN PHARM CO.,LTD
XINXING TOWN, ZHUCHENG, SHANDONG 262218 CHINA
TEL: 0086.536.6349756 FAX: 0086.536.6523666

ZCHT 浩天药业 HAOTIAN PHARMA

DIETHYL ETHER



GENERAL INFORMATION

CAS REGISTRY NUMBER:	60-29-7
MOLECULAR FORMULA:	$(C_2H_5)_2O$
MOLECULAR WEIGHT:	74.12

CHEMICAL AND PHYSICAL REQUIREMENTS

APPEARANCE:	COLORLESS LIQUID
ODOUR	ANESTHETIC
PURITY:	>95.0
BOILING POINT:	34.6°C
PEROXIDE	<1 PPM

PACKAGING, SAFETY AND HANDLING

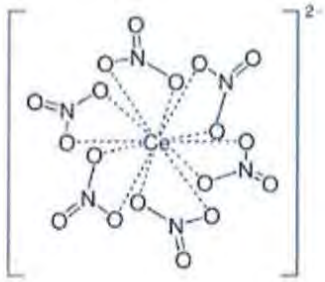
PACKAGING:	UMTAMPERED CYLINDER
STORAGE:	IN DRY SPACE AT REFRIGERATED CONDITIONS
SHELF LIFE	1 YEAR
SAFETY:	EXTREMELY FLAMMABLE, VAPOR INHALATION MAY CAUSE HEADACHE, NAUSEA, VOMITING AND LOST OF CONSCIOUSNESS. FULL PREVENTATIVE GOWNING IS NECESSARY WHEN HANDLING

APPROVED SUPPLIER(S)

XIAMEN HISUNNY CHEMICAL COMPANY
HEFEI TNJ CHEMICAL INDUSTRY COMPANY

ZHUCHENG HAOTIAN PHARM CO..LTD
XINXING TOWN, ZHUCHENG, SHANDONG 262218 CHINA
TEL: 0086.536.6349756 FAX: 0086.536.6523666

AMMONIUM CERIC NITRATE



GENERAL INFORMATION

CAS REGISTRY NUMBER:	16774-21-3
MOLECULAR FORMULA:	$H_8CeN_8O_{18}$
MOLECULAR WEIGHT:	548.23

CHEMICAL AND PHYSICAL REQUIREMENTS

APPEARANCE:	ORANGE-YELLOW CRYSTALLINE POWDER
PURITY:	>98.0%
MELTING POINT:	108°C
LOSS ON DRYING	<5.0%
TOTAL HEAVY METALS as Pb	<10 ppm

PACKAGING, SAFETY AND HANDLING

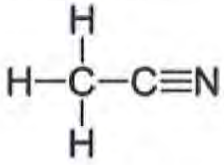
PACKAGING:	10, 25KG FIBER DRUM
STORAGE:	IN DRY SPACE AT AMBIENT CONDITIONS
SHELF LIFE	1 YEAR
SAFETY:	STRONG OXIDING AGENT; MAY CAUSE COMBUSTION AND FIRE WHEN IN CONTACT WITH OTHER SUBSTANCES. IT MAY CAUSE IRRITATION TO THE EYES, SKIN, AND THE RESPIRATORY TRACT.

APPROVED SUPPLIER(S)

SHANGHAI SHENHAO COMPANY
TCI CHEMICALS

ZCHT 浩天药业 HAOTIAN PHARMA

ACETONITRILE



GENERAL INFORMATION

CAS REGISTRY NUMBER:	75-05-8
MOLECULAR FORMULA:	CH_3CN
MOLECULAR WEIGHT:	41.05

CHEMICAL AND PHYSICAL REQUIREMENTS

APPEARANCE:	COLORLESS LIQUID
ODOUR	PUNGENT
PURITY:	>99.0%
BOLING POINT:	81.3-82.1°C
FLASH POINT	6°C

PACKAGING, SAFETY AND HANDLING

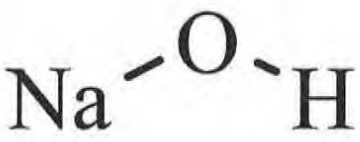
PACKAGING:	25KG
STORAGE:	IN DRY SPACE AT REFRIGERATED CONDITION
SHELF LIFE	1 YEAR
SAFETY:	FLAMMABLE, FULL PREVENTATIVE GOWNING IS NECESSART WHEN HANDLING

APPROVED SUPPLIER(S)

SHANGHAI TITANCHEM COMPANY
TCI CHEMICALS COMPANY

ZCHT 浩天药业 HAOTIAN PHARMA

SODIUM HYDROXIDE



GENERAL INFORMATION

CAS REGISTRY NUMBER:	1310-73-2
MOLECULAR FORMULA:	NaOH
MOLECULAR WEIGHT:	39.997

CHEMICAL AND PHYSICAL REQUIREMENTS

APPEARANCE:	WHITE OPAQUE FLAKES
PURITY:	>99.0%
MELTING POINT:	318°C
ASH	<0.2%
TOTAL HEAVY METALS as Pb	<10 ppm

PACKAGING, SAFETY AND HANDLING

PACKAGING:	25KG POLYLINED BAG
STORAGE:	IN DRY SPACE AT AMBIENT CONDITIONS
SHELF LIFE	1 YEAR
SAFETY:	CORROSIVE FULL PREVENTATIVE GOWNING IS NECESSARY WHEN HANDLING

APPROVED SUPPLIER(S)

TIANJIN YUANLONG CHEMICAL COMPANY
NANJING YUNDUN CHEMICALS COMPANY

ZHUCHENG HAOTIAN PHARM CO..LTD
XINXING TOWN, ZHUCHENG, SHANDONG 262218 CHINA
TEL: 0086.536.6349756 FAX: 0086.536.6523666

SODIUM CHLORIDE

$Na^+ Cl^-$

GENERAL INFORMATION

CAS REGISTRY NUMBER:	7647-14-5
MOLECULAR FORMULA:	NaCl
MOLECULAR WEIGHT:	58.44

CHEMICAL AND PHYSICAL REQUIREMENTS

APPEARANCE:	COLORLESS CRYSTALS
PURITY:	>95.0%
ASH	<0.2%
TOTAL HEAVY METALS as Pb	<10 ppm

PACKAGING, SAFETY AND HANDLING

PACKAGING:	25KG POLYLINED BAG
STORAGE:	IN DRY SPACE AT AMBIENT CONDITIONS
SHELF LIFE	2 YEAR
SAFETY:	MAY CAUSE IRRITATION TO THE EYES

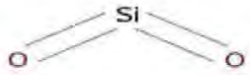
APPROVED SUPPLIER(S)

MULTIPLE

ZHUCHENG HAOTIAN PHARM CO..LTD
XINXING TOWN, ZHUCHENG, SHANDONG 262218 CHINA
TEL: 0086.536.6349756 FAX: 0086.536.6523666

ZCHT 浩天药业 HAOTIAN PHARMA

SILICON DIOXIDE



GENERAL INFORMATION

CAS REGISTRY NUMBER:	7631-86-9
MOLECULAR FORMULA:	SiO_2
MOLECULAR WEIGHT:	60.08

CHEMICAL AND PHYSICAL REQUIREMENTS

APPEARANCE:	WHITE CRYSTALLINE POWDER
PURITY:	>95.0%
LOSS ON DRYING	<7%
ASH	<10%
TOTAL HEAVY METALS as Pb	<10 ppm

PACKAGING, SAFETY AND HANDLING

PACKAGING:	25KG POLYLINED BAG
STORAGE:	IN DRY SPACE AT AMBIENT CONDITIONS
SHELF LIFE	2 YEAR
SAFETY:	N/A

APPROVED SUPPLIER(S)

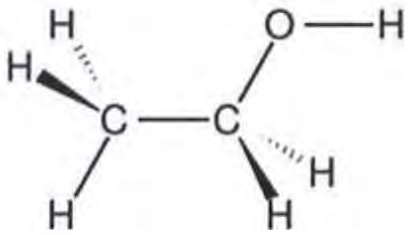
W.R. GRACE
HENAN TIANFU CHEMICAL COMPANY
SHOUGUANG BAOTE CHEMICALS

ZHUCHENG HAOTIAN PHARM CO..LTD
XINXING TOWN, ZHUCHENG, SHANDONG 262218 CHINA
TEL: 0086.536.6349756 FAX: 0086.536.6523666

PURCHASING SPECIFICATION - RAW MATERIAL

ZCHT 浩天药业 HAOTIAN PHARMA

ETHYL ALCOHOL



GENERAL INFORMATION

CAS REGISTRY NUMBER:	64-17-5
MOLECULAR FORMULA:	CH_3CH_3OH
MOLECULAR WEIGHT:	46.07

CHEMICAL AND PHYSICAL REQUIREMENTS

APPEARANCE:	CLEAR LIQUID
PURITY:	>99.0
BOILING POINT:	78.37°C

PACKAGING, SAFETY AND HANDLING

PACKAGING:	55 GALLON METAL DRUMS
STORAGE:	IN DRY SPACE AT AMBIENT CONDITIONS
SHELF LIFE	1 YEAR
SAFETY:	MAY CAUSES SEVERE EYE IRRITATION

APPROVED SUPPLIER(S)

WUXI XINHUI CHEMICAL COMPANY
SHANGHAI JIULIN INDUSTRIAL COMPANY

ZHUCHENG HAOTIAN PHARM CO..LTD
XINXING TOWN, ZHUCHENG, SHANDONG 262218 CHINA
TEL: 0086.536.6349756 FAX: 0086.536.6523666

Raw Material Risk Analysis–

All materials that are used in the production of PQQ are high purity chemicals with low risk level of microbiological contamination. The incoming raw material testing and material handling are governed by SMP-QC-002-01 and SMP-M-01701.

Ingredient List	Chemical Structure	CAS Number	Purity	Potential Risk(s)	Source	Risk Mitigation / Measures
Ethyl 6-amino-5 Methoxy 1H Indole-2-carboxylate	$C_{12}H_{14}N_2O_3$	107575-60-0	>97.0%	N/A	N/A	N/A
Dimethyl Oxoglutaconate	$C_7H_8O_5$	78939-37-4	>95.0%	N/A	N/A	N/A
Dichloromethane	CH_2Cl_2	75-09-2	>99.0%	Heavy Metals	Supplier Manufacturing Process	filtration and purification
Copper Acetate Monohydrate	$Cu(OAc)_2.H_2O$	6046-93-1	>99.0%	Heavy Metals	Supplier manufacturing Process	filtration and purification Final product analysis
Hydrogen Chloride (g)	HCL	7647-01-0	>99.0%	N/A	N/A	N/A
Sodium Hydrogen Carbonate	$NaHCO_3$	144-55-8	>99.0%	Heavy Metals Sulfur Compounds	Supplier manufacturing Process	filtration and purification
Diethyl Ether	$(C_2H_5)_2O$	60-29-7	>96.0%	Hydrocarbons Peroxide	Supplier manufacturing Process	filtration and purification
Ceric Ammonium Nitrate	$H_8CeN_8O_{18}$	16774-21-3	>98.0%	Heavy Metals	Supplier Manufacturing Process	Filtration and purification
Acetonitrile	CH_3CN	75-05-8	>98.0%	Carbonyl compounds	Supplier manufacturing Process	filtration and purification
Sodium Hydroxide	NaOH	1310-73-2	>97.0%	Heavy Metals	Supplier manufacturing Process	filtration and purification
Sodium Chloride	NaCl	7647-14-5	>99.0%	N/A	N/A	N/A
Silicon Dioxide	SiO_2	7631-86-9	>99.0%	N/A	N/A	N/A
Ethanol	CH_3CH_2OH	64-17-5	>99.0%	N/A	N/A	N/A
Purified Water	H_2O			Micro-organism	Self-supplied	Test before usage

APPENDIX 4

Product Specifications and Certificates of Analysis for PQQ

PRODUCT SPECIFICATION

ZCHT 浩天药业 HAOTIAN PHARMA

PYRROLOQUINOLINE QUINONE DISODIUM SALT (PQQ)

PRODUCT DESCRIPTION:	A stable and water-soluble reddish brown crystalline powder with mild hydroscopic property that is an essential micronutrient for human body, heart and mind. Recommended daily dose: 10mg-20mg

ITEMS	**PARAMETERS**	**METHODS**	**RESULTS**
SENSORY REQUIREMENTS			
APPEARANCE	CRYSTALLINE POWDER	VISUAL	CONFORMITY
COLOR	REDDISH BROWN	VISUAL	CONFORMITY
TASTE	SALTY	TASTE	CONFORMITY
PHYSICAL - CHEMICAL REQUIREMENTS			
IDENTIFICATION	MATCH STANDARD	FTIR	CONFORMITY
ASSAY (DRY BASIS)	NOT LESS THAN 98%	HPLC	REPORT VALUE
LOSS ON DRYING	NOT MORE THAN 12%	USP<731>	REPORT VALUE
PARTICLE SIZE	NOT LESS THAN 99% THROUGH 20 MESH	USP<786>	REPORT VALUE
ASH*	NOT MORE THAN 1.0%	USP<281>	REPORT VALUE
CONTAMINANTS/ADDITIVES REQUIREMENTS			
TOTAL HEAVY METALS as Pb	NOT MORE THAN 10 PPM	USP<231>	REPORT VALUE
ARSENIC (As)	NOT MORE THAN 1.0 PPM	AAS	REPORT VALUE
CADMIUM (Cd)	NOT MORE THAN 1.0 PPM	AAS	REPORT VALUE
LEAD (Pb)	NOT MORE THAN 0.5 PPM	AAS	REPORT VALUE
MERCURY (Hg)	NOT MORE THAN 0.1 PPM	AAS	REPORT VALUE
RESIDUAL SOLVENT (ETHANOL)	NOT MORE THAN 0.5%	USP<467>	REPORT VALUE
MICROBIOLOGICAL REQUIREMENTS*			
AEROBIC PLATE COUNT	NOT MORE THAN 1000 cfu/g	USP<61>	REPORT VALUE
YEAST & MOLD	NOT MORE THAN 100 cfu/g	USP<61>	REPORT VALUE
E.COLI	NEGATIVE	USP<61>	CONFORMITY
SALMONELLA	NEGATIVE/10G	USP<61>	CONFORMITY

PACKAGING, STORAGE & TRANSPORTATION	
PACKAGING	1KG or 5KG IN SEALED FOIL BAG, IMPERVIOUS TO LIGHT AND HUMIDITY WITH A SEALED INNER BAG.
STORAGE	UNTAMPERED PACKAGE CAN BE STORED UNDER ROOM CONDITIONS; REFRIGERATION IS PREFERRED.
SHELF LIFE	2 YEARS
COUNTRY OF ORIGIN	CHINA

*TEST COULD BE PERFORMED ONLY ON PERIODIC BASES.

Version 02, Revision 01

GLOBAL MARKET REPRESENTATIVE
NASCENT HEALTH SCIENCES
E-MAIL: INFO@NASCENT-HEALTH.COM
WWW.SOPURESTEVIA.COM

MANUFACTURER
ZHUCHENG HAOTIAN PHARM CO., LTD
XINXING TOWN, ZHUCHENG, SHANDONG 262218 CHINA
TEL: 0086.536.6349756 FAX:0086.536.6352646
WWW.ZCHT.CC

ZCHT 浩天药业 HAOTIAN PHARMA

PYRROLOQUINOLINE QUINONE DISODIUM SALT (PQQ)

CERTIFICATE OF ANALYSIS

LOT NUMBER: 20110413

MANUFACTURED DATE: 2011.04.13

ISSUED DATE: 2011.04.19

TEST ITEMS	PARAMETERS	RESULTS
IDENTIFICATION	MATCH STANDARD	POSITIVE
APPEARANCE	CRYSTALLINE POWDER	CONFORMS
COLOR	REDDISH BROWN	CONFORMS
TASTE	SALTY	CONFORMS
ASSAY (DRY BASIS)	NOT LESS THAN 98.0%	99.0%
LOSS ON DRYING	NOT MORE THAN 12%	8.7%
HEAVY METALS as Pb	NOT MORE THAN 10 PPM	<10 PPM
RESIDUAL SOLVENT (ETHANOL, %)	NOT MORE THAN 0.5	0.1%
COUNTRY OF ORIGIN	CHINA	

TESTED BY：莫金科　　CHECKED BY：曹建军　　ISSUED BY：

COA Version 01, Revision 01

ZHUCHENG HAOTIAN PHARM CO..LTD
XINXING TOWN, ZHUCHENG, SHANDONG 262218 CHINA
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ZCHT 浩天药业 HAOTIAN PHARMA

PYRROLOQUINOLINE QUINONE DISODIUM SALT (PQQ)

CERTIFICATE OF ANALYSIS

LOT NUMBER: 201301011

MANUFACTURED DATE: 2013.01.31

ISSUED DATE: 2013.02.01

TEST ITEMS	PARAMETERS	RESULTS
IDENTIFICATION	MATCH STANDARD	POSITIVE
APPEARANCE	CRYSTALLINE POWDER	CONFORMS
COLOR	REDDISH BROWN	CONFORMS
TASTE	SALTY	CONFORMS
ASSAY (DRY BASIS)	NOT LESS THAN 98.0%	99.3%
LOSS ON DRYING	NOT MORE THAN 12%	5.0%
HEAVY METALS as Pb	NOT MORE THAN 10 PPM	<10 PPM
RESIDUAL SOLVENT (ETHANOL, %)	NOT MORE THAN 0.5	0.13%
PACKAGING	IN SEALED FOIL BAG, IMPERVIOUS TO LIGHT AND HUMIDITY, WITH A SEALED INNER BAG.	
STORAGE	SEALED PACKAGE CAN BE STORED UNDER ROOM CONDITIONS, REFRIGERATION IS PREFERRED.	
COUNTRY OF ORIGIN	CHINA	

TESTED BY：莫金科　　CHECKED BY：曹建军　　ISSUED BY：

COA Version 01, Revision 02
Revision Date: 01/01/2013

ZHUCHENG HAOTIAN PHARM CO..LTD
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ZCHT 浩天药业 HAOTIAN PHARMA

PYRROLOQUINOLINE QUINONE DISODIUM SALT (PQQ)

CERTIFICATE OF ANALYSIS

ITEM NUMBER:	004	LOT NUMBER:	201306003
MANUFACTURE DATE:	2013.06.11	EXPIRATION DATE:	2015.06.10

TEST ITEMS	PARAMETERS	RESULTS
IDENTIFICATION	MATCH STANDARD	POSITIVE
APPEARANCE	CRYSTALLINE POWDER	CONFORMS
COLOR	REDDISH BROWN	CONFORMS
TASTE	SALTY	CONFORMS
ASSAY (DRY BASIS)	NOT LESS THAN 98%	99.5%
LOSS ON DRYING	NOT MORE THAN 12%	3.4%
PARTICLE SIZE	NOT LESS THAN 99% THROUGH 20 MESH	99.5%
ASH	NOT MORE THAN 1.0%	0.05%
HEAVY METALS as Pb	NOT MORE THAN 10 PPM	<10 PPM
ARSENIC (As)	NOT MORE THAN 1.0 PPM	NOT DETECTED
CADMIUM (Cd)	NOT MORE THAN 1.0 PPM	0.03 PPM
LEAD (Pb)	NOT MORE THAN 0.5 PPM	NOT DETECTED
MERCURY (Hg)	NOT MORE THAN 0.1 PPM	NOT DETECTED
RESIDUAL SOLVENT (ETHANOL, %)	NOT MORE THAN 0.5	0.12
AEROBIC PLATE COUNT	NOT MORE THAN 1000 cfu/g	<10 cfu/g
YEAST & MOLD	NOT MORE THAN 100 cfu/g	<10 cfu/g
E.COLI	NEGATIVE/G	NEGATIVE
SALMONELLA	NEGATIVE/10G	NEGATIVE
PACKAGING	IN SEALED FOIL BAG, IMPERVIOUS TO LIGHT AND HUMIDITY, WITH A SEALED INNER BAG.	
STORAGE	SEALED PACKAGE CAN BE STORED UNDER ROOM CONDITIONS, REFRIGERATION IS PREFERRED.	
COUNTRY OF ORIGIN	CHINA	

TESTED BY：莫金科　　CHECKED BY：曹建军　　ISSUED BY：

COA Version 02, Revision 01
Revision Date: 06/01/2013

ZHUCHENG HAOTIAN PHARM CO..LTD
XINXING TOWN, ZHUCHENG, SHANDONG 262218 CHINA
TEL: 0086.536.6349756 FAX: 0086.536.6523666

ZCHT 浩天药业 HAOTIAN PHARMA

PYRROLOQUINOLINE QUINONE DISODIUM SALT (PQQ)

CERTIFICATE OF ANALYSIS

ITEM NUMBER:	004	LOT NUMBER:	201310001
MANUFACTURE DATE:	2013.10.29	EXPIRATION DATE:	2015.10.28

TEST ITEMS	**PARAMETERS**	**RESULTS**
APPEARANCE	CRYSTALLINE POWDER	CONFORMS
COLOR	REDDISH BROWN	CONFORMS
TASTE	SALTY	CONFORMS
IDENTIFICATION	POSITIVE MATCH	CONFORMS
ASSAY (DRY BASIS)	NOT LESS THAN 98%	99.1%
LOSS ON DRYING	NOT MORE THAN 12%	4.8%
PARTICLE SIZE	NOT LESS THAN 99% THROUGH 20 MESH	99.8%
ASH	NOT MORE THAN 1.0%	0.05%
HEAVY METALS as Pb	NOT MORE THAN 10 PPM	<10 PPM
ARSENIC (As)	NOT MORE THAN 1.0 PPM	NOT DETECTED
CADMIUM (Cd)	NOT MORE THAN 1.0 PPM	0.02 PPM
LEAD (Pb)	NOT MORE THAN 0.5 PPM	NOT DETECTED
MERCURY (Hg)	NOT MORE THAN 0.1 PPM	NOT DETECTED
RESIDUAL SOLVENT (ETHANOL, %)	NOT MORE THAN 0.5	0.1
AEROBIC PLATE COUNT	NOT MORE THAN 1000 cfu/g	80
YEAST & MOLD	NOT MORE THAN 100 cfu/g	<10
E.COLI	NEGATIVE/1G	NEGATIVE
SALMONELLA	NEGATIVE/10G	NEGATIVE
PACKAGING	IN SEALED FOIL BAG, IMPERVIOUS TO LIGHT AND HUMIDITY, WITH A SEALED INNER BAG.	
STORAGE	SEALED PACKAGE CAN BE STORED UNDER ROOM CONDITIONS, REFRIGERATION IS PREFERRED.	
COUNTRY OF ORIGIN	CHINA	

TESTED BY：莫金科　　CHECKED BY：曹建军　　ISSUED BY：

COA Version 02, Revision 01
Revision Date: 06/01/2013

ZHUCHENG HAOTIAN PHARM CO..LTD
XINXING TOWN, ZHUCHENG, SHANDONG 262218 CHINA
TEL: 0086.536.6349756 FAX: 0086.536.6523666

PYRROLOQUINOLINE QUINONE DISODIUM SALT (PQQ)

CERTIFICATE OF ANALYSIS

ITEM NUMBER:	004	LOT NUMBER:	201310002
MANUFACTURE DATE:	2013.10.31	EXPIRATION DATE:	2015.10.30

TEST ITEMS	**PARAMETERS**	**RESULTS**
APPEARANCE	CRYSTALLINE POWDER	CONFORMS
COLOR	REDDISH BROWN	CONFORMS
TASTE	SALTY	CONFORMS
IDENTIFICATION	POSITIVE MATCH	CONFORMS
ASSAY (DRY BASIS)	NOT LESS THAN 98%	99.1%
LOSS ON DRYING	NOT MORE THAN 12%	4.6%
PARTICLE SIZE	NOT LESS THAN 99% THROUGH 20 MESH	99.5%
ASH	NOT MORE THAN 1.0%	0.05%
HEAVY METALS as Pb	NOT MORE THAN 10 PPM	<10 PPM
ARSENIC (As)	NOT MORE THAN 1.0 PPM	NOT DETECTED
CADMIUM (Cd)	NOT MORE THAN 1.0 PPM	0.01 PPM
LEAD (Pb)	NOT MORE THAN 0.5 PPM	NOT DETECTED
MERCURY (Hg)	NOT MORE THAN 0.1 PPM	NOT DETECTED
RESIDUAL SOLVENT (ETHANOL, %)	NOT MORE THAN 0.5	0.2
AEROBIC PLATE COUNT	NOT MORE THAN 1000 cfu/g	<10
YEAST & MOLD	NOT MORE THAN 100 cfu/g	<10
E.COLI	NEGATIVE/1G	NEGATIVE
SALMONELLA	NEGATIVE/10G	NEGATIVE
PACKAGING	IN SEALED FOIL BAG, IMPERVIOUS TO LIGHT AND HUMIDITY, WITH A SEALED INNER BAG.	
STORAGE	SEALED PACKAGE CAN BE STORED UNDER ROOM CONDITIONS, REFRIGERATION IS PREFERRED.	
COUNTRY OF ORIGIN	CHINA	

TESTED BY：莫金科　　CHECKED BY：曹建军　　ISSUED BY：

COA Version 02, Revision 01
Revision Date: 06/01/2013

ZHUCHENG HAOTIAN PHARM CO..LTD
XINXING TOWN, ZHUCHENG, SHANDONG 262218 CHINA
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PYRROLOQUINOLINE QUINONE DISODIUM SALT (PQQ)

CERTIFICATE OF ANALYSIS

ITEM NUMBER:	004	LOT NUMBER:	201310003
MANUFACTURE DATE:	2013.11.03	EXPIRATION DATE:	2015.11.02

TEST ITEMS	**PARAMETERS**	**RESULTS**
APPEARANCE	CRYSTALLINE POWDER	CONFORMS
COLOR	REDDISH BROWN	CONFORMS
TASTE	SALTY	CONFORMS
IDENTIFICATION	POSITIVE MATCH	CONFORMS
ASSAY (DRY BASIS)	NOT LESS THAN 98%	99.1%
LOSS ON DRYING	NOT MORE THAN 12%	5.0%
PARTICLE SIZE	NOT LESS THAN 99% THROUGH 20 MESH	99.5%
ASH	NOT MORE THAN 1.0%	0.05%
HEAVY METALS as Pb	NOT MORE THAN 10 PPM	<10 PPM
ARSENIC (As)	NOT MORE THAN 1.0 PPM	NOT DETECTED
CADMIUM (Cd)	NOT MORE THAN 1.0 PPM	0.01 PPM
LEAD (Pb)	NOT MORE THAN 0.5 PPM	NOT DETECTED
MERCURY (Hg)	NOT MORE THAN 0.1 PPM	NOT DETECTED
RESIDUAL SOLVENT (ETHANOL, %)	NOT MORE THAN 0.5	0.1
AEROBIC PLATE COUNT	NOT MORE THAN 1000 cfu/g	<10
YEAST & MOLD	NOT MORE THAN 100 cfu/g	<10
E.COLI	NEGATIVE/1G	NEGATIVE
SALMONELLA	NEGATIVE/10G	NEGATIVE
PACKAGING	IN SEALED FOIL BAG, IMPERVIOUS TO LIGHT AND HUMIDITY, WITH A SEALED INNER BAG.	
STORAGE	SEALED PACKAGE CAN BE STORED UNDER ROOM CONDITIONS, REFRIGERATION IS PREFERRED.	
COUNTRY OF ORIGIN	CHINA	

TESTED BY：莫金科　　CHECKED BY：曹建军　　ISSUED BY：

COA Version 02, Revision 01
Revision Date: 06/01/2013

ZHUCHENG HAOTIAN PHARM CO..LTD
XINXING TOWN, ZHUCHENG, SHANDONG 262218 CHINA
TEL: 0086.536.6349756 FAX: 0086.536.6523666

SGS

测试报告 No: SHFDN130100098FD 报告日期: 2013-01-11

客户名称: 潍坊盛瑜药业有限公司
客户地址: 山东省寿光市羊口镇环渤海精细化工园内

样品由客户提供及申请者对样品的说明(除 SGS 相关号、SGS 工作号、样品接收日期、样品测试日期外)如下:

样品名称: 吡咯喹啉醌二钠盐
批号/生产日期:004201212001
生产商: 潍坊盛瑜药业有限公司
SGS 相关号: SHAFD1300255801
SGS 工作号: SHFDN130100098FD
样品接收日期: 2013-01-06
样品测试日期: 2013-01-06 ~ 2013-01-10

SAMPLE OF CERIUM (Ce)

TEST REPORT

测试要求:
根据申请者的要求:
理化测试: 铈

测试方法:
SGS 实验室方法---ICP/MS

测试结果:

测试项目	测试方法	测试结果	方法检出限
铈 mg/kg	实验室方法	未检出	1

样品描述:袋装物

SGS CONSUMER TESTING SERVICES SHANGHAI
SGS 授权签字人

*** 报告结束***

通标标准技术服务(上海)有限公司检测中心
第 1 页 共 1 页 RAND: 2371013

SHCM 4958951

SGS-CSTC Standards Technical Services (Shanghai) Co.,Ltd.
Testing Center
3rd Building,No.889 Yishan Road Xuhui District,Shanghai China 200233
中国·上海·徐汇区宜山路889号3号楼 邮编: 200233
t E&E (86-21) 61402553 f E&E (86-21)64953679 www.sgsgroup.com.cn
HL: (86-21) 61402594 HL: (86-21)54500353 e sgs.china@sgs.com

报 告 编 号：**SIMM-07-11~13**
Certificate No.

检 测 报 告
Certificate of Analysis

中国科学院上海药物研究所测试实验室
Analytical Laboratory
Shanghai Institute of Materia Medica
Chinese Academy of Sciences

机构检测专用章
Special Test seal

(b) (6)

批准人_____
Approved by

报告日期 2015 年 11 月 27 日
Date for report Year Month Day

声　　明
STATEMENTS

1. 本报告提供的结果仅对本次被测的样品有效。
   The results in this report are only valid for the sample received.

2. 未经本实验室批准，部分采用本报告内容无效。
   Adopting parts of the report is invalid without any approval by our laboratory.

3. 自检测报告发出之日起算，本实验室受理检测质量申诉的有效期限为 15 天。
   The complaint limitation of re-test is 15 days from the date the report is issued.

4. 样品保存期限一般为 1 个月。稳定性较差的样品不予保存。
   Samples are preserved for a month normally, except for those with poor stability.

5. 若有任何疑问或咨询，请通过以下方式进行联系
   If you have any question or advice, please contact us through the following ways:

中国科学院上海药物研究所测试实验室
Analytical Laboratory Shanghai Institute of Materia Medica Chinese Academy of Sciences

地址：上海市浦东新区张江祖冲之路 555 号,邮编：201203
Address：555 Rd. Zuchongzhi, Shanghai, China, 201203

电话(Tel)：0086-21-5080 6600 转(ext.) 5202 或(or) 5206

传真(Fax)：0086-21-5080 6053

邮箱(Email)：dychen@simm.ac.cn

联系人：陈东英 研究员
Contact Person: Dong-ying Chen, PhD, Prof.

报 告 编 号：SIMM-07-11~13

Certificate No.

样品编号 Sample No.	SIMM-07-11~13
样品名称 Sample Name	吡咯并喹啉醌二钠盐 Pyrroloquinoline Quinone Disodium Salt
批　　号 Batch No.	004201306001、004201306002、004201306003
规　　格 Specification	/
样品状态描述 Sample Description	红棕色粉末 Reddish brown Powder
委托单位 Sample Submitted By	诸城市浩天药业有限公司 Zhucheng Haotian Pharmaceutical Co., Ltd.
委托单位地址 Company Address	中国山东省诸城市辛兴镇浩天路 1 号 No. 1 Haotian Road, Xinxing Town Zhucheng City, Shandong Province, China
实验方法 Method	气相色谱法 GC-FID
检测仪器 Instrument	Agilent 6890 气相色谱仪 Agilent 6890 Gas Chromatograph
收样日期 Receive date	2013 年 06 月 19 日 June 19th, 2013
检测日期 Test date	2013 年 08 月 22 日~2013 年 09 月 13 日 August 22nd, 2013 ~ September 13th, 2013
备注 Remarks	/

样品批号：004201306001 **报告编号：SIMM-07-11**

Batch No. Certificate No.

检测结果

Results of test

检验项目 Determination	标准规定 Specification	结果 Result
Residual Acetonitrile	Not more than 410ppm	Not Detected
Residual Acetone	Not more than 5000ppm	Not Detected
Residual Ethanol	Not more than 5000ppm	8ppm
Residual Ether	Not more than 5000ppm	Not Detected
Residual Ethyl acetate	Not more than 5000ppm	Not Detected
Residual Ethyl formate	Not more than 5000ppm	Not Detected
Residual Hexane	Not more than 290ppm	Not Detected
Residual Methanol	Not more than 3000ppm	Not Detected
Residual Methylene chloride	Not more than 600ppm	Not Detected
Residual Toluene	Not more Than 890ppm	Not Detected

检测结果内容结束

End of Report

样品批号：004201306002 **报告编号：SIMM-07-12**

Batch No. Certificate No.

检测结果

Results of test

检验项目 Determination	标准规定 Specification	结果 Result
Residual Acetonitrile	Not more than 410ppm	Not Detected
Residual Acetone	Not more than 5000ppm	Not Detected
Residual Ethanol	Not more than 5000ppm	22ppm
Residual Ether	Not more than 5000ppm	Not Detected
Residual Ethyl acetate	Not more than 5000ppm	Not Detected
Residual Ethyl formate	Not more than 5000ppm	Not Detected
Residual Hexane	Not more than 290ppm	Not Detected
Residual Methanol	Not more than 3000ppm	Not Detected
Residual Methylene chloride	Not more than 600ppm	Not Detected
Residual Toluene	Not more Than 890ppm	Not Detected

检测结果内容结束

End of Report

样品批号：004201306003 **报告编号：SIMM-07-13**

Batch No. Certificate No.

检测结果

Results of test

检验项目 Determination	标准规定 Specification	结果 Result
Residual Acetonitrile	Not more than 410ppm	Not Detected
Residual Acetone	Not more than 5000ppm	Not Detected
Residual Ethanol	Not more than 5000ppm	33ppm
Residual Ether	Not more than 5000ppm	Not Detected
Residual Ethyl acetate	Not more than 5000ppm	Not Detected
Residual Ethyl formate	Not more than 5000ppm	Not Detected
Residual Hexane	Not more than 290ppm	Not Detected
Residual Methanol	Not more than 3000ppm	Not Detected
Residual Methylene chloride	Not more than 600ppm	Not Detected
Residual Toluene	Not more Than 890ppm	Not Detected

检测结果内容结束

End of Report

Standard solution—Prepare a solution in N,N-dimethyl formamide containing 300 μg of methanol, 500 μg of ethanol, acetone, ethyl acetate, ethyl formate, ether, 60 μg of methylene chloride, 90 μg of toluene, 40 μg of acetonitrile and 25 μg of hexane per mL.

Test solution—A 0.2 g accurately weighed portion of the material is dissolved in 2 mL N,N-dimethyl formamide.

Chromatographic system-The gas chromatograph is equipped with a flame-ionization detector, a 0.53-mm × 60-m fused silica analytical column with a 3-μm chemically cross-linked 6% cyanopropylphenyl / 94% dimethyl polysiloxane stationary phase. The injection port temperature and the detector temperature are maintained at 180° (with 10:1 split ratio) and 250°, respectively. Nitrogen was used as carrier gas the constant pressure of 3.0 psi. The column temperature is programmed as follows. Initially, the column temperature is maintained at 40° for 20 min, then increased at a rate of 10° per minute to 200 ° and maintained at 200° for 12 min. The headspace oven temperature, loop and transfer line temperatures are maintained at 90°, 120° and 130°, respectively. The injection volume is 1000 μL.

Detection limit: Methanol, 4.5 ppm; Ethanol, 5.0 ppm; Acetone, 2.9 ppm; Ethyl Acetate, 2.9 ppm; Ethyl formate, 5.1 ppm; Ether, 0.8 ppm; Methylene Chloride, 15.1 ppm; Toluene, 2.3 ppm; Acetonitrile, 10.1 ppm; Hexane, 0.3 ppm.

APPENDIX 5

Assay for PQQ by HPLC

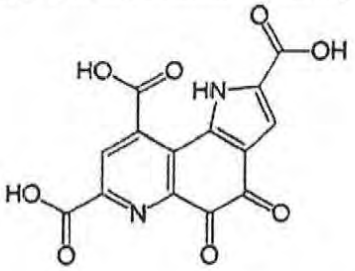
TITLE: **Assay for PQQ Pyrroloquinonline Quinone by HPLC**

1. **OBJECTIVE:**
To determine Pyrroloquinonline Quinone (PQQ) content in Raw materials using Reverse Phase HPLC

2. **INSTRUMENTATION:**
WATERS Alliance 2690 Separation Module
Detector: Waters PDA 996

3. **PRINCIPLE/METHOD DESCRIPTION:**
Standard and sample solutions in the concentration of 0.08 mg/ml are prepared in DI Water. Aliquots of the standard and sample solution are treated with 0.1M sodium carbonate buffer pH 9.20 and reacted with Acetone to give a stable adduct which is then quantitated on Reverse Phases HPLC using a C18 column with UV Detector at 254 nm.

4. **CHEMICAL STRUCTURES/CHEMISTRY:**
Molecular nature of PQQ: Because there are three carboxyl groups, PQQ is a water-soluble compound. It has a quinone skeleton as well as vitamin B2 (riboflavin), and the part of left side contains a similar chemical property to that of vitamin B6.



Pyrroloquinoline quinone

Synonym:	Methoxatin, PQQ, 4,5-Dihydro-4,5-dioxo-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylic acid
CAS Number:	72909-34-3
Linear Formula:	$C_{14}H_6O_68N_2O_8$
Molecular Weight:	330.206 g/mol

5. **REFERENCE STANDARD:**

5.1. Standard Pyrroloquinoline quinone ~98% (HPLC) Catalog # D-7783 from Sigma Aldrich
http://www.sigmaaldrich.com/catalog/product/sigma/d7783?lang=en&region=US

5.2. Alternately the Standard for Pyrroloquinoline quinone can also be purchased from Santa Cruz Biotechnology Catalog # sc-210178
http://www.scbt.com/datasheet-210178-Pyrroloquinoline-quinone.html

6. **SOLVENTS & REAGENTS:**
6.1. Water, HPLC grade
6.2. Acetonitrile, HPLC Grade
6.3. Acetone, ACS Reagent Grade
6.4. Glacial Acetic Acid
6.5. Sodium carbonate

6.6. Concentrated Nitric acid
6.7. **16 % Acetone:** Prepare by pipetting 16ml Acetone and transferring to 100 ml volumetric flask. QS to volume with DI Water. Mix well.
6.8. **0.1 M Sodium Carbonate Buffer (pH 9.2):** Prepare by weighing 1.2 g of sodium carbonate and transfer to 100 ml volumetric flask, add 30 ml DI Water and stir to dissolve. Cautiously pipette in 0.6 ml of Concentrated Nitric Acid using micropipette. QS to volume with DI Water, Check pH and adjust to 9.2 with Diluted Nitric acid or 0.1 N NaOH. Do not keep more than a week, check pH before use.

7. STANDARD PREPARATION:

7.1. **STOCK SOLUTION:** Transfer 10 mg accurately weighed reference standard into a 25ml volumetric flask.
7.2. Dissolve in 10 ml DI Water by sonication for 5 minutes.
7.3. Cool to room temperature and QS to volume with DI Water.
7.4. Refrigerate prepared standard till use.
7.5. **WORKING STANDARD:** Quantatively pipette and transfer 1 ml from stock to a 5 ml volumetric flask.
7.6. Pipette 1ml of the 0.1 M Sodium carbonate buffer solution.
7.7. QS to volume with 3 ml of 16 % Acetone solution and mix well.
7.8. Place in warm water bath (37° C) for 30 minutes,
7.9. Cool to room temperature.
7.10. Filter through 0.45Micron filter into HPLC vial.
7.11. Final standard concentration is 0.08 mg/ml

NOTE: If using 1 mg Reference standard, prepare accordingly to have working concentration of 0.08 mg/ml

8. SAMPLE PREPARATION:

8.1. Weigh accurately a powdered sample equivalent to 40 mg of PQQ into a 100ml volumetric flask.
8.2. Dissolve in 60ml DI Water by sonication for 10 minutes
8.3. Cool to RT and QS to volume with DI Water.
8.4. Quantatively pipette and transfer 1 ml to a 5 ml volumetric flask.
8.5. Pipette 1ml of the 0.1 M Sodium carbonate buffer solution.
8.6. QS to volume with 3 ml of 16 % Acetone solution and mix well.
8.7. Place in warm water bath (37° C) for 30 minutes,
8.8. Cool to room temperature.
8.9. Filter through 0.45Micron filter into HPLC vial.

9. ANALYTICAL METHOD:

9.1. **Diluting Solution:** DI Water, 16 % Acetone and 0.1 M Sodium Carbonate Buffer (pH 9.2)
9.2. **Mobile Phase A: 0.1% Glacial Acetic acid in Purified Water :**Prepare 1000 ml at a time, Pipette 1 ml of Glacial Acetic Acid to 999 ml of HPLC Grade Deionized water, Stir/Mix well for 20 minutes, Filer and degas before use
9.3. **Mobile Phase B: 0.1% Glacial acetic Acid in HPLC-Grade Acetonitrile:** Prepare 1000 ml at a time, Pipette 1 ml of Glacial Acetic Acid to 999 ml of HPLC Grade Acetonitrile, Stir/Mix well for 20 minutes, Filer and degas before use
9.4. **Mobile Phase Program**: 70: 30 (A:B)
9.5. **Column**: Agilent Zorbax Eclipse XDB C18, 5um, 4.6 X 150 mm, packing L1 Part # 993967

9.6. **Column Temperature** Ambient (Room Temperature)
9.7. **Detector:** UV @ 254 nm
9.8. **Flow Rate**: 0.5 ml/min
9.9. **Injection Volume:** 10 µl
9.10. **Run Time**:10 minutes
9.11. **Retention times:**

5.4.1	PQQ:	2.46 minutes
5.4.2	Solvent (Acetone):	3.3 minutes

6 CALCULATION:

Calculate the amount of PQQ in the sample by using the formula:

Ru/Rs X Cs/Cu X P X 100/Spl wt, where

Ru: Peak AREA response of the sample (Average 2 injections)
Rs: Peak AREA response of the standard (Average 5 injections)
Cs: Concentration of Standard (in mg/ml)
Cu: Concentration of sample
P: Purity of the Standard
100: To calculate in percentage
Spl wt: The weight of the sample taken in mg

7 CHROMATOGRAMS:

7.4 **System Suitability**: The Relative Standard Deviation for Replicate Injections is not more than 2.0 %

8 ATTACHMENT:

8.4 Chromatographic raw data for 5 standard injections with summary followed by sample injections with summary and a solvent injection.

Component Summary For Retention Time
Channel: 996

	SampleName	Inj	Channel	Vial	PQQ	Acetone
1	PQQ Std	1	996	20	2.456	3.318
2	PQQ Std	2	996	20	2.462	3.303
3	PQQ Std	3	996	20	2.462	3.298
4	PQQ Std	4	996	20	2.467	3.315
5	PQQ Std	5	996	20	2.461	3.296
Mean					2.462	3.306
Std. Dev.					0.004	0.010
% RSD					0.2	0.3

Component Summary For Area
Channel: 996

	SampleName	Inj	Channel	Vial	PQQ	Acetone
1	PQQ Std	1	996	20	4895746	5009246
2	PQQ Std	2	996	20	4851843	4825408
3	PQQ Std	3	996	20	4853486	4742426
4	PQQ Std	4	996	20	4881456	4779766
5	PQQ Std	5	996	20	4839748	4651885
Mean					4864456	4801746
Std. Dev.					23227	132360
% RSD					0.5	2.8

Component Summary For Amount
Channel: 996

	SampleName	Inj	Channel	Vial	PQQ	Acetone
1	PQQ Std	1	996	20		
2	PQQ Std	2	996	20		
3	PQQ Std	3	996	20		
4	PQQ Std	4	996	20		
5	PQQ Std	5	996	20		

Reported by User: System

Project Name: Phytochemicals4

Component Summary For Amount
Channel: 996

	SampleName	Inj	Channel	Vial	PQQ	Acetone
Mean						
Std. Dev.						
% RSD						

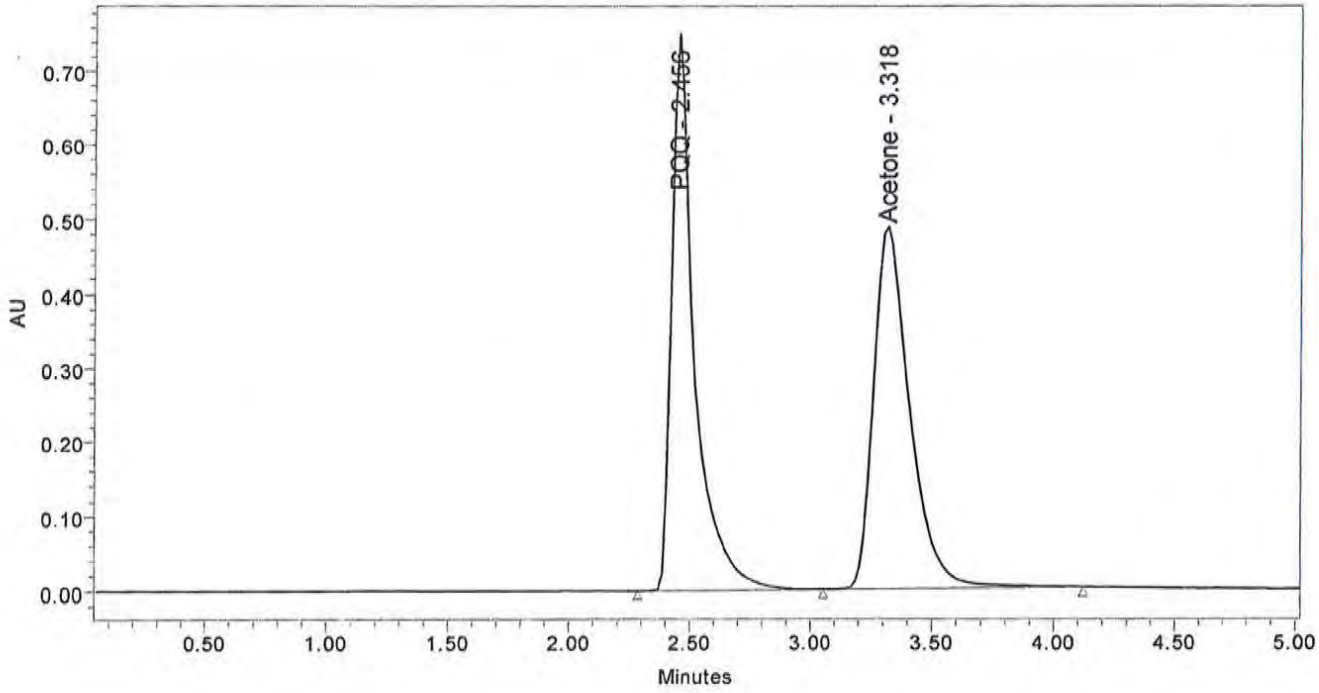
Default Individual Report

Reported by User: System

Project Name: Phytochemicals4

SAMPLE INFORMATION

Sample Name:	PQQ Std	Acquired By:	System
Sample Type:	Standard	Date Acquired:	6/19/2012 12:30:05 PM
Vial:	20	Acq. Method Set:	PQQ
Injection #:	1	Date Processed:	6/19/2012 1:31:01 PM
Injection Volume:	10.00 ul	Processing Method:	PQQ
Run Time:	5.0 Minutes	Channel Name:	Extract 254.0
Sample Set Name:	061912 PQQ	Proc. Chnl. Descr.:	PDA 254.0 nm



	Peak Name	RT	Area	% Area	Height
1	PQQ	2.456	4895746	49.43	740909
2	Acetone	3.318	5009246	50.57	486581

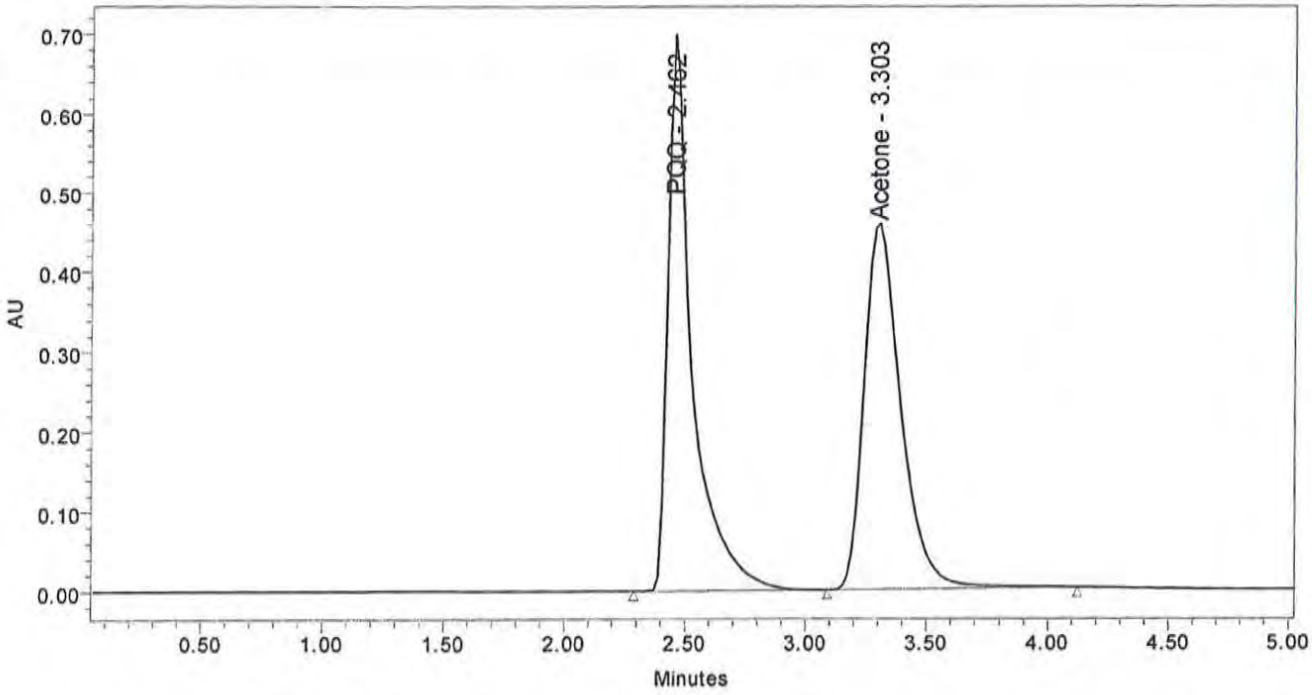
Default Individual Report

Reported by User: System

Project Name: Phytochemicals4

SAMPLE INFORMATION

Sample Name:	PQQ Std	Acquired By:	System
Sample Type:	Standard	Date Acquired:	6/19/2012 12:36:01 PM
Vial:	20	Acq. Method Set:	PQQ
Injection #:	2	Date Processed:	6/19/2012 1:31:05 PM
Injection Volume:	10.00 ul	Processing Method:	PQQ
Run Time:	5.0 Minutes	Channel Name:	Extract 254.0
Sample Set Name:	061912 PQQ	Proc. Chnl. Descr.:	PDA 254.0 nm



	Peak Name	RT	Area	% Area	Height
1	PQQ	2.462	4851843	50.14	691956
2	Acetone	3.303	4825408	49.86	459462

Empower software

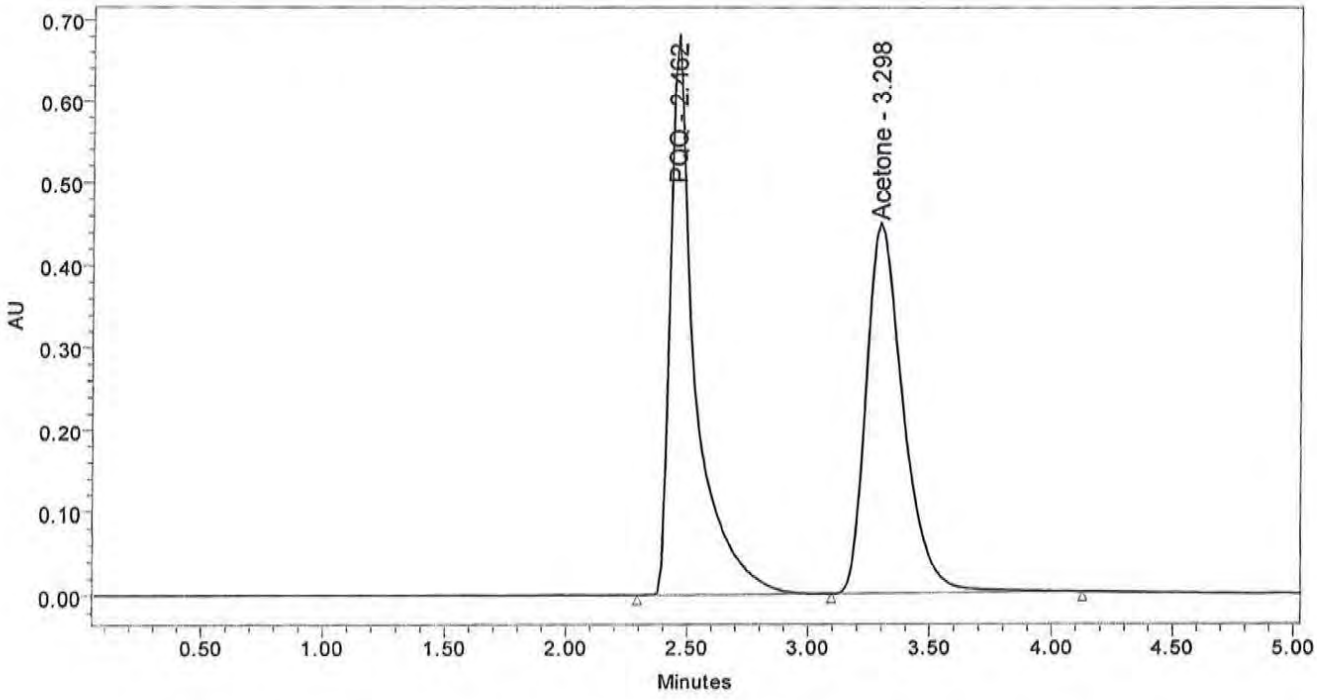
Default Individual Report

Reported by User: System

Project Name: Phytochemicals4

SAMPLE INFORMATION

Sample Name:	PQQ Std	Acquired By:	System
Sample Type:	Standard	Date Acquired:	6/19/2012 12:41:44 PM
Vial:	20	Acq. Method Set:	PQQ
Injection #:	3	Date Processed:	6/19/2012 1:31:08 PM
Injection Volume:	10.00 ul	Processing Method:	PQQ
Run Time:	5.0 Minutes	Channel Name:	Extract 254.0
Sample Set Name:	061912 PQQ	Proc. Chnl. Descr.:	PDA 254.0 nm



	Peak Name	RT	Area	% Area	Height
1	PQQ	2.462	4853486	50.58	672119
2	Acetone	3.298	4742426	49.42	449927

Empower software

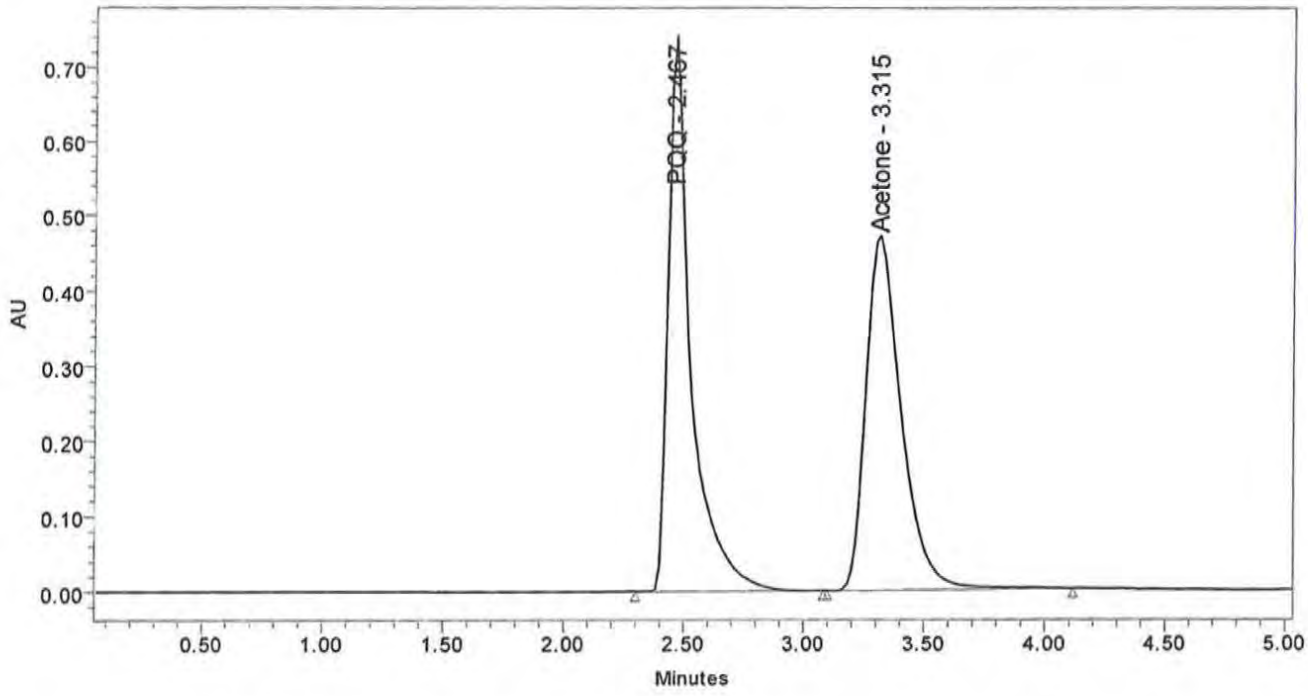
Default Individual Report

Reported by User: System

Project Name: Phytochemicals4

SAMPLE INFORMATION

Sample Name:	PQQ Std	Acquired By:	System
Sample Type:	Standard	Date Acquired:	6/19/2012 12:47:30 PM
Vial:	20	Acq. Method Set:	PQQ
Injection #:	4	Date Processed:	6/19/2012 1:31:11 PM
Injection Volume:	10.00 ul	Processing Method:	PQQ
Run Time:	5.0 Minutes	Channel Name:	Extract 254.0
Sample Set Name:	061912 PQQ	Proc. Chnl. Descr.:	PDA 254.0 nm



	Peak Name	RT	Area	% Area	Height
1	PQQ	2.467	4881456	50.53	730122
2	Acetone	3.315	4779766	49.47	470538

Empower software

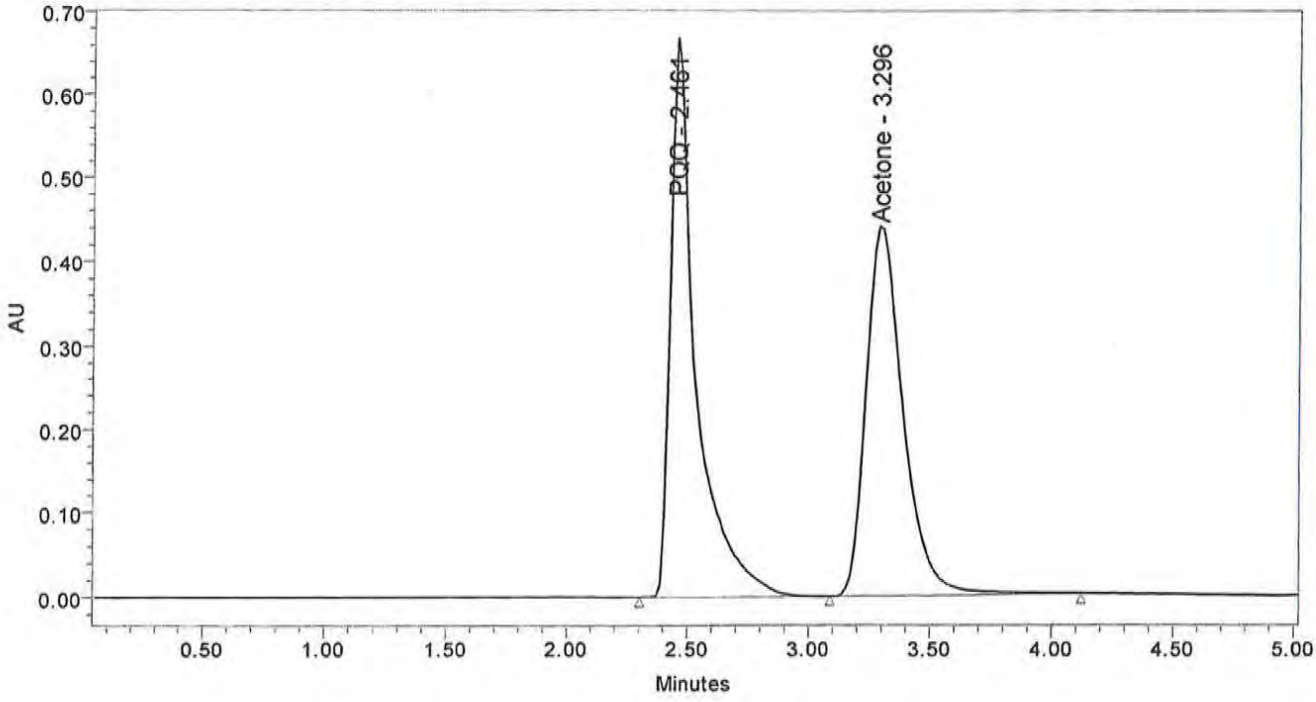
Default Individual Report

Reported by User: System

Project Name: Phytochemicals4

SAMPLE INFORMATION

Sample Name:	PQQ Std	Acquired By:	System
Sample Type:	Standard	Date Acquired:	6/19/2012 12:53:13 PM
Vial:	20	Acq. Method Set:	PQQ
Injection #:	5	Date Processed:	6/19/2012 1:31:14 PM
Injection Volume:	10.00 ul	Processing Method:	PQQ
Run Time:	5.0 Minutes	Channel Name:	Extract 254.0
Sample Set Name:	061912 PQQ	Proc. Chnl. Descr.:	PDA 254.0 nm



	Peak Name	RT	Area	% Area	Height
1	PQQ	2.461	4839748	50.99	665152
2	Acetone	3.296	4651885	49.01	443247

Component Summary For Retention Time
Channel: 996

	SampleName	Inj	Channel	Vial	PQQ	Acetone
1	CH 4120 12	1	996	21	2.459	3.305
2	CH 4120 12	2	996	21	2.474	3.311
Mean					2.466	3.308
Std. Dev.					0.011	0.005
% RSD					0.4	0.1

Component Summary For Area
Channel: 996

	SampleName	Inj	Channel	Vial	PQQ	Acetone
1	CH 4120 12	1	996	21	4839630	4654785
2	CH 4120 12	2	996	21	4834157	4574726
Mean					4836893	4614756
Std. Dev.					3871	56610
% RSD					0.1	1.2

Component Summary For Amount
Channel: 996

	SampleName	Inj	Channel	Vial	PQQ	Acetone
1	CH 4120 12	1	996	21		
2	CH 4120 12	2	996	21		
Mean						
Std. Dev.						
% RSD						

Empower software

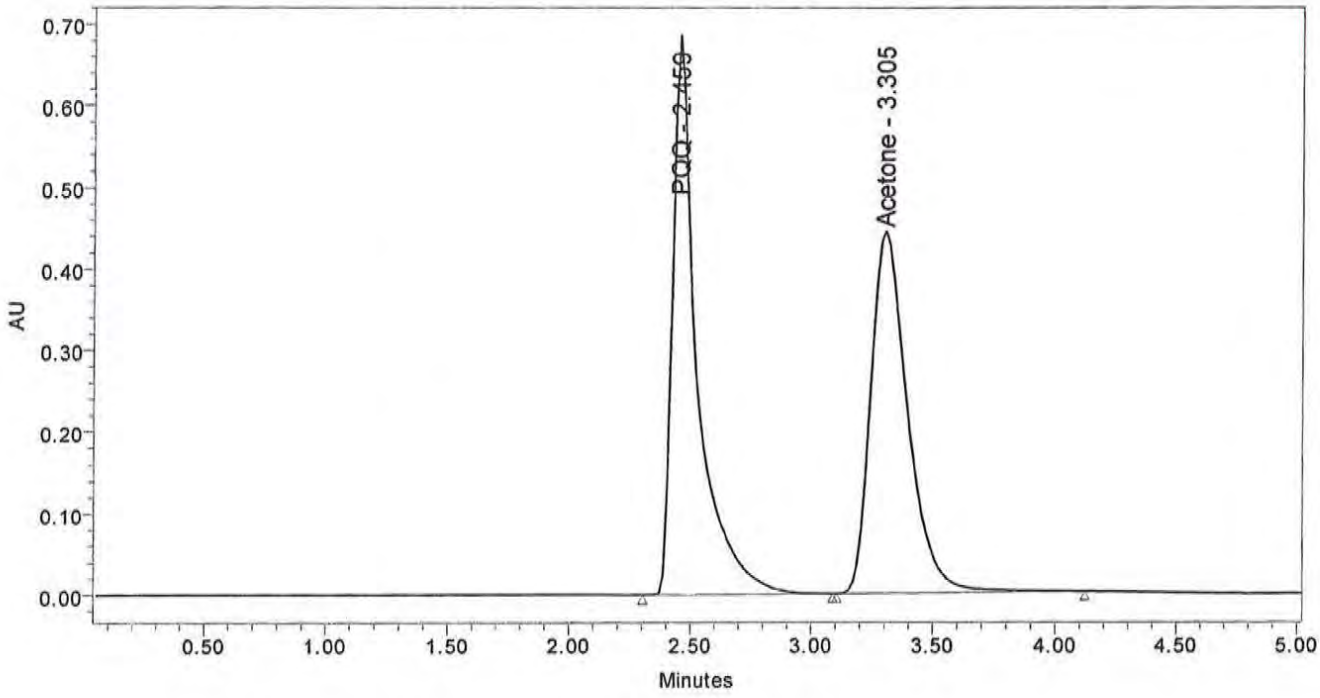
Default Individual Report

Reported by User: System

Project Name: Phytochemicals4

SAMPLE INFORMATION

Sample Name:	CH 4120 12	Acquired By:	System
Sample Type:	Unknow n	Date Acquired:	6/19/2012 12:59:04 PM
Vial:	21	Acq. Method Set:	PQQ
Injection #:	1	Date Processed:	6/19/2012 1:31:18 PM
Injection Volume:	10.00 ul	Processing Method:	PQQ
Run Time:	5.0 Minutes	Channel Name:	Extract 254.0
Sample Set Name:	061912 PQQ	Proc. Chnl. Descr.:	PDA 254.0 nm



	Peak Name	RT	Area	% Area	Height
1	PQQ	2.459	4839630	50.97	678087
2	Acetone	3.305	4654785	49.03	443343

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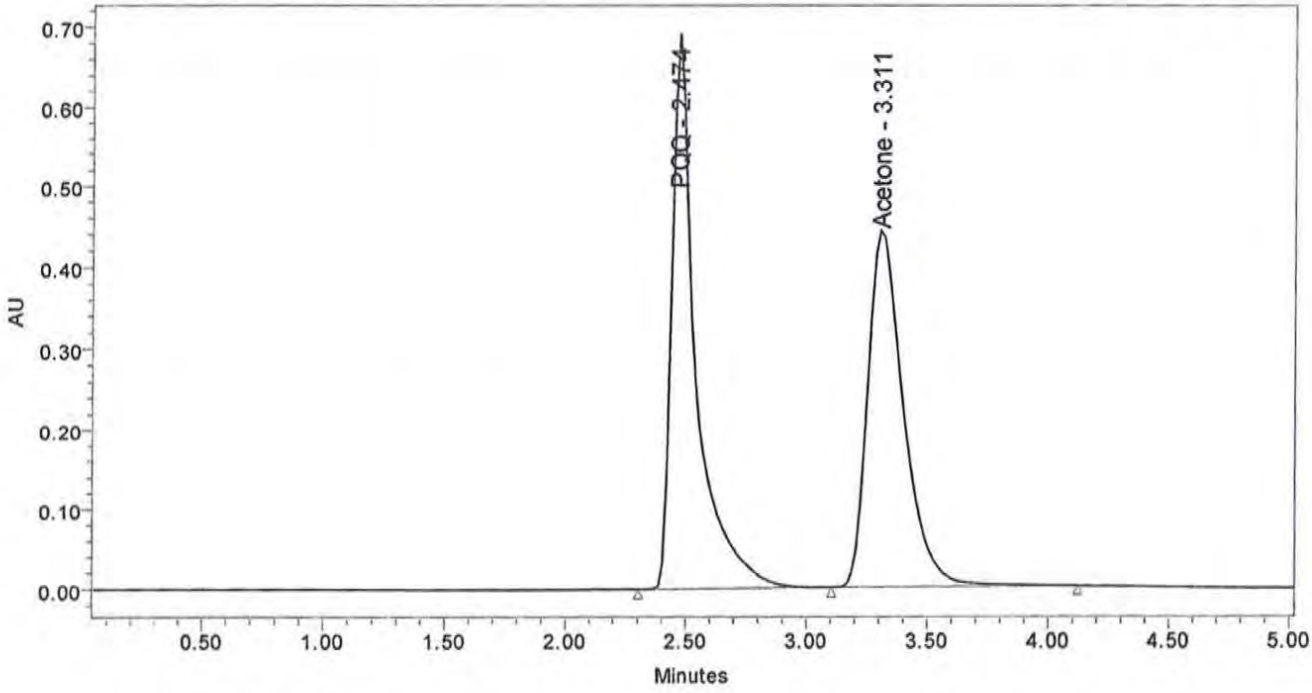
Default Individual Report

Reported by User: System

Project Name: Phytochemicals4

SAMPLE INFORMATION

Sample Name:	CH 4120 12	Acquired By:	System
Sample Type:	Unknow n	Date Acquired:	6/19/2012 1:04:48 PM
Vial:	21	Acq. Method Set:	PQQ
Injection #:	2	Date Processed:	6/19/2012 1:31:21 PM
Injection Volume:	10.00 ul	Processing Method:	PQQ
Run Time:	5.0 Minutes	Channel Name:	Extract 254.0
Sample Set Name:	061912 PQQ	Proc. Chnl. Descr.:	PDA 254.0 nm



	Peak Name	RT	Area	% Area	Height
1	PQQ	2.474	4834157	51.38	682587
2	Acetone	3.311	4574726	48.62	444703

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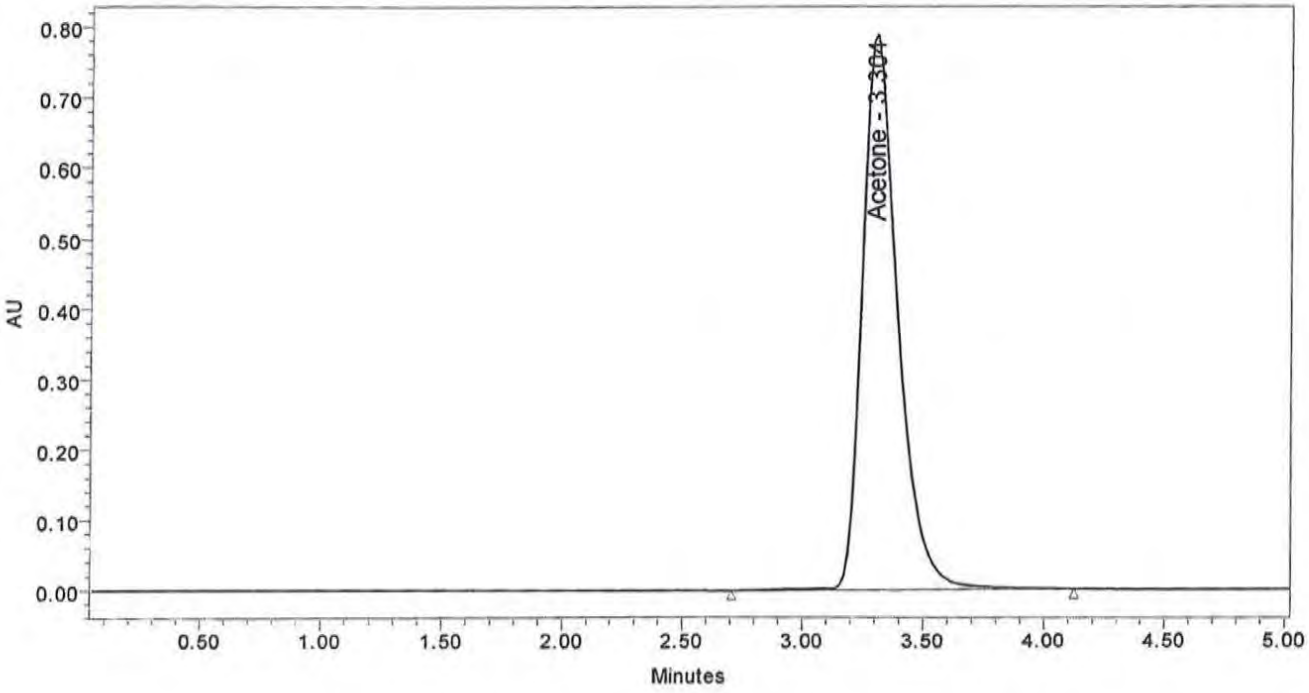
Default Individual Report

Reported by User: System

Project Name: Phytochemicals4

SAMPLE INFORMATION

Sample Name:	16% Acetone	Acquired By:	System
Sample Type:	Control	Date Acquired:	6/19/2012 1:16:26 PM
Vial:	23	Acq. Method Set:	PQQ
Injection #:	1	Date Processed:	6/19/2012 1:31:34 PM
Injection Volume:	10.00 ul	Processing Method:	PQQ
Run Time:	5.0 Minutes	Channel Name:	Extract 254.0
Sample Set Name:	061912 PQQ	Proc. Chnl. Descr.:	PDA 254.0 nm



	Peak Name	RT	Area	% Area	Height
1	PQQ	2.467			
2	Acetone	3.304	8005857	100.00	789533

APPENDIX 6

Estimated Daily Intake of PQQ Disodium Salt by the U.S. Population from Proposed Food-Uses

Estimated Daily Intake of Pyrroloquinoline Quinone Disodium Salt by the United States Population from Proposed Uses in Beverages

Prepared for: Nascent Health Sciences, LLC
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Allentown, New Jersey
08501

Prepared by: Intertek Scientific & Regulatory Consultancy
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Mississauga, Ontario, Canada
L5N 2X7
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January 26, 2016

Estimated Daily Intake of Pyrroloquinoline Quinone Disodium Salt by the United States Population from Proposed Uses in Beverages

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Estimated Daily Intake of Pyrroloquinoline Quinone Disodium Salt by the United States Population from Proposed Uses in Beverages

1.0 INTRODUCTION

Pyrroloquinoline quinone (PQQ) disodium salt is proposed for use in the United States (U.S.) in beverages such as energy, sport, or isotonic drinks, non-milk based meal replacement beverages, and bottled, enhanced, or fortified waters.

Estimates for the intake of PQQ disodium salt were based on the proposed beverage-uses and use-levels for PQQ disodium salt in conjunction with food consumption data included in the U.S. National Center for Health Statistics' (NCHS) National Health and Nutrition Examination Surveys (NHANES) 2011-2012 (CDC, 2014; USDA, 2014). Calculations for the mean and 90th percentile all-person and all-user intakes were performed for each of the individual proposed beverage-uses of PQQ disodium salt and the percentage of consumers were determined. Similar calculations were used to estimate the total intake of PQQ disodium salt resulting from all proposed beverage-uses of PQQ disodium salt combined. In both cases, the per person and per kilogram body weight intakes were reported for the following population groups:

- Infants and young children, ages 0 to 2[1];
- Children, ages 3 to 11;
- Female teenagers, ages 12 to 19;
- Male teenagers, ages 12 to 19;
- Female adults, ages 20 and up;
- Male adults, ages 20 and up; and
- Total population (all age and gender groups combined).

2.0 FOOD CONSUMPTION SURVEY DATA

2.1 Survey Description

NHANES for the years 2011-2012 are available for public use. NHANES are conducted as continuous, annual surveys, and are released in 2-year cycles. Each year about 7,000 people

[1] • PQQ disodium salt is not intended for use in infant formula and infant foods and products containing it will not be marketed directly to this consumer group.

from 15 different locations across the U.S. are interviewed, and approximately 5,000 complete the health examination component of the survey. Any combination of consecutive years of data collection is recognized and used as a nationally representative sample of the U.S. population. It is well-established that the length of a dietary survey affects the estimated consumption of individual users and that short-term surveys, such as a 1-day dietary survey, may overestimate consumption compared to surveys conducted over longer time periods (Anderson, 1988). Because two 24-hour dietary recalls administered on 2 non-consecutive days are available from the NHANES 2011-2012 survey, these data were used to generate estimates for the current intake analysis.

NHANES 2011-2012 survey data were collected from individuals and households *via* 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2) throughout all 4 seasons of the year. Day 1 data were collected in-person, and Day 2 data were collected by telephone in the following 3 to 10 days, on different days of the week, to achieve the desired degree of statistical independence. The data were collected by first selecting Primary Sampling Units (PSUs), which were counties throughout the U.S., of which 15 PSUs are visited per year. Small counties were combined to attain a minimum population size. These PSUs were segmented and households were chosen within each segment. One or more participants within a household were interviewed. For NHANES 2011-2012, 13,431 individuals were selected for the sample, 9,756 were interviewed (72.6%) and 9,338 were sampled (69.5%).

In addition to collecting information on the types and quantities of foods being consumed, NHANES 2011-2012 collected socio-economic, physiological and demographic information from individual participants in the survey, such as sex, age, height and weight, and other variables useful in characterizing consumption. The inclusion of this information allows for further assessment of food intake based on consumption by specific population groups of interest within the total population. The sample design for NHANES 2011-2012 includes an oversample of Asian Americans, however sample weights were incorporated to allow estimates from these subgroups to be combined to obtain national estimates that reflect the relative proportions of these groups in the population as a whole (CDC, 2014; USDA, 2014).

2.2 Statistical Methods

Statistical analysis and data management were conducted in DaDiet Software (Dazult Ltd., 2015). DaDiet Software is a web-based software tool that allows accurate estimate of exposure to nutrients and to substances added to foods, including contaminants, food additives and novel ingredients. The main input components are concentration (use level) data and food consumption data. Data sets are combined in the software to provide accurate and efficient exposure assessments.

For the intake assessment, consumption data from individual dietary records, detailing food items ingested by each survey participant, were collated by computer and used to generate estimates for the intake of PQQ disodium salt by the U.S. population using DaDiet Software. Estimates for the daily intake of PQQ disodium salt represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES 2011-2012 data; these average amounts comprised the distribution from which mean and percentile intake estimates were generated. Mean and percentile estimates were generated incorporating survey weights in order to provide representative intakes for the entire U.S. population. All-person intake refers to the estimated intake of PQQ disodium salt averaged over all individuals surveyed, regardless of whether they potentially consumed beverage products containing PQQ disodium salt, and therefore includes individuals with "zero" intakes (*i.e*. those who reported no intake of beverage products containing PQQ disodium salt during the 2 survey days). All-user intake refers to the estimated intake of PQQ disodium salt by those individuals who reported consuming beverage products containing PQQ disodium salt, hence the "all-user" designation. Individuals were considered "users" if they consumed 1 or more food products containing PQQ disodium salt on either Day 1 or Day 2 of the survey.

Mean and 90th percentile intake estimates based on sample sizes of less than 30 and 80, respectively, may not be considered statistically reliable due to the limited sampling size (LSRO, 1995). As such, the reliability of estimates for the intake of PQQ disodium salt based on the consumption of these beverages may be questionable for certain individual population groups and are marked with an asterisk in Appendices A and B.

3.0 FOOD USAGE DATA

The individual proposed beverage-uses and use-levels for PQQ disodium salt employed in the current intake analysis are summarized in Table 3-1. Food codes representative of each proposed beverage-use were chosen from the NHANES 2011-2012 (CDC, 2014; USDA, 2014). Food codes were grouped in use categories according to Title 21, Section §170.3 of the Code of Federal Regulations (U.S. FDA, 2015a). Product-specific adjustment factors were developed based on data provided in the standard recipe file for the Continuing Survey of Food Intakes by Individuals (CSFII) 1994-1996, 1998 survey (USDA, 2000). All food codes included in the current intake assessment are listed in Appendix C.

Table 3-1 Summary of the Individual Proposed Beverage-Uses and Use-Levels for PQQ Disodium Salt in the U.S. (2011-2012 NHANES Data)				
Food Category	**Beverage-Uses**	**PQQ Disodium Salt Level (mg/serving)**	**RACC* (g or mL)**	**Use-Levels (mg/g)**
Beverages and Beverage Bases	Energy, Sport, and Isotonic Drinks	8	240	0.0333
	Non-Milk Based Meal Replacement Beverages	8	240	0.0333
	Water (Bottled, Enhanced, Fortified)	8	240	0.0333

NHANES = National Health and Nutrition Examination Survey; PQQ = pyrroloquinoline quinone; U.S. = United States.
* RACC = Reference Amounts Customarily Consumed per Eating Occasion (21 CFR §101.12 – U.S. FDA, 2015b).

4.0 FOOD SURVEY RESULTS

Estimates for the total daily intakes of PQQ disodium salt from proposed beverage-uses are provided in Tables 4.1-1 and 4.1-2. Estimates for the daily intake of PQQ disodium salt from individual proposed beverage-uses in the U.S. are summarized in Tables A-1 to A-7 and B-1 to B-7 of Appendices A and B, respectively. Tables A-1 to A-7 provide estimates for the daily intake of PQQ disodium salt per person (mg/day), whereas Tables B-1 to B-7 provide estimates for the daily intake of PQQ disodium salt on a per kilogram body weight basis (mg/kg body weight/day).

4.1 Estimated Daily Intake of PQQ Disodium Salt from All Proposed Beverage-Uses in the U.S.

Table 4.1-1 summarizes the estimated total intake of PQQ disodium salt (mg/person/day) from all proposed beverage-uses in the U.S. population group. Table 4.1-2 presents this data on a per kilogram body weight basis (mg/kg body weight/day). The percentage of users was greater than 33.4% of the population groups, with female teens having the greatest percentage of users at 65.8% (Table 4.1-1). Low user percentages within a population group typically lead to dissimilar results for the all-person and all-user consumption estimates; therefore, only the all-user intakes will be discussed in further detail as they represent the exposures expected for the consumer (target) population.

Among consumers in the total population, the mean and 90th percentile all-user intakes of PQQ disodium salt were determined to be 26.5 and 61.4 mg/person/day, respectively. Of the individual population groups, male adults were determined to have the greatest mean and 90th percentile all-user intakes of PQQ disodium salt on an absolute basis, at 32.7 and 69.7 mg/person/day, respectively, while infants and young children had the lowest mean and 90th percentile all-user intakes of 8.4 and 20.8 mg/person/day, respectively (Table 4.1-1).

Table 4.1-1 Summary of the Estimated Daily Intake of PQQ Disodium Salt from Proposed Beverage-Uses in the U.S. by Population Group (2011-2012 NHANES Data)

Population Group	Age Group (Years)	All-Person Consumption (mg/day)		All-Users Consumption (mg/day)			
		Mean	90th Percentile	% Users	n	Mean	90th Percentile
Infants	0 to 2	2.8	9.3	33.4	254	8.4	20.8
Children	3 to 11	6.1	18.9	49.7	858	12.2	27.0
Female Teenagers	12 to 19	13.7	35.6	65.8	334	20.8	42.9
Male Teenagers	12 to 19	14.8	41.5	60.1	314	24.7	58.3
Female Adults	20 and up	14.3	43.8	50.6	1,209	28.2	63.1
Male Adults	20 and up	15.4	52.2	47.0	1,091	32.7	69.7
Total Population	All Ages	13.2	41.6	50.0	4,060	26.5	61.4

NHANES = National Health and Nutrition Examination Survey; PQQ = pyrroloquinoline quinone; U.S. = United States.

On a body weight basis, the mean and 90th percentile all-person intakes in the total population were determined to be 0.2 and 0.6 mg/kg body weight/day, respectively. Among consumers, the mean and 90th percentile all-user intakes for all age groups were 0.4 and 0.9 mg/kg body weight/day, respectively. Infants and young children were identified as having the highest mean and 90th percentile all-user intakes of any population group, of 0.7 and 1.5 mg/kg body weight/day, respectively. However, it is noted that infants and young children do not represent the target consumer population for products containing PQQ disodium salt and this is thus an overestimation of actual intakes expected in this age category. Female teens had the lowest mean and 90th percentile all-user intakes of 0.3 and 0.7 mg/kg body weight/day, respectively (Table 4.1-2).

Table 4.1-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of PQQ Disodium Salt from Proposed Beverage-Uses in the U.S. by Population Group (2011-2012 NHANES Data)

Population Group	Age Group (Years)	All-Person Consumption (mg/kg bw/day)		All-Users Consumption (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants	0 to 2	0.2	0.8	33.4	253	0.7	1.5
Children	3 to 11	0.2	0.7	49.7	858	0.4	1.0
Female Teenagers	12 to 19	0.2	0.6	66.0	326	0.3	0.7
Male Teenagers	12 to 19	0.2	0.6	60.1	313	0.4	0.8
Female Adults	20 and up	0.2	0.6	50.5	1,194	0.4	0.8
Male Adults	20 and up	0.2	0.6	46.9	1,079	0.4	0.9
Total Population	All Ages	0.2	0.6	49.8	4,023	0.4	0.9

bw = body weight; NHANES = National Health and Nutrition Examination Survey; PQQ = pyrroloquinoline quinone; U.S. = United States.

4.2 Estimated Daily Intake of PQQ Disodium Salt from Individual Proposed Beverage-Uses in the U.S.

Estimates for the mean and 90th percentile daily intakes of PQQ disodium salt from each individual food category are summarized in Tables A-1 to A-7 and B-1 to B-7 on a mg/day and mg/kg body weight/day basis, respectively. The total U.S. population was identified as being significant consumers of bottled, enhanced, and fortified water (31.1 to 60.8% users) and energy, sport, and isotonic drinks (3.7 to 24.5% users).

In terms of contribution to total mean intake of PQQ disodium salt, bottled, enhanced, and fortified water contributed 79.1 to 93.7% to total mean intakes, whereas non-milk based meal replacements contributed to less than 3.3% across all age categories (see Tables A-1 to A-7 and/or B-1 to B-7 for further details).

5.0 CONCLUSIONS

Consumption data and information pertaining to the individual proposed beverage-uses of PQQ disodium salt were used to estimate the all-person and all-user intakes of PQQ disodium salt for specific demographic groups and for the total U.S. population. This type of intake methodology is generally considered to be "worst case" as a result of several conservative assumptions made in the consumption estimates. For example, it is often assumed that all food products within a food category contain the ingredient at the maximum specified level of use. In addition, it is well-established that the length of a dietary survey affects the estimated consumption of

individual users. Short-term surveys, such as the typical 2- or 3-day dietary surveys, may overestimate the consumption of food products that are consumed relatively infrequently.

In summary, on an all-user basis, the mean and 90th percentile intakes in the consuming U.S. population were estimated to be 26.5 mg/person/day (0.4 mg/kg body weight/day) and 61.4 mg/person/day (0.9 mg/kg body weight/day), respectively. On a body weight basis, the mean and 90th percentile intake of PQQ disodium salt among teenagers and adults were 0.3 to 0.4 mg/kg body weight/day and 0.7 to 0.9 mg/kg body weight/day, respectively. Infants and young children (aged 2 years and under), who are not representative of the target population, had the highest mean and 90th percentile intake on a per body weight basis at 0.7 and 1.5 mg/kg body weight/day, respectively.

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Appendix A
Estimated Daily Intake of PQQ Disodium Salt from Individual Proposed Beverage-Uses by Different Population Groups Within the U.S.

Table A-1 Estimated Daily Intake of PQQ Disodium Salt from Individual Proposed Beverage-Uses by Infants and Young Children Aged 0 to 2 Years Within the U.S. (2011-2012 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	All-Person Consumption (mg/day)		All-Users Consumption (mg/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***2.8***	***9.3***	***33.4***	***254***	***8.4***	***20.8***
Beverages and Beverage Bases							
Energy, Sport, and Isotonic Drinks	10.4	0.3*	na	3.7	29	7.8*	26.8*
Non-Milk Based Meal Replacement Beverages	3.3	0.1*	na	1.2	7	8.0*	7.7*
Water (Bottled, Enhanced, Fortified)	86.4	2.4	8.4	31.1	237	7.8	17.8

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

Table A-2 Estimated Daily Intake of PQQ Disodium Salt from Individual Proposed Beverage-Uses by Children Aged 3 to 12 Years Within the U.S. (2011-2012 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	All-Person Consumption (mg/day)		All-Users Consumption (mg/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***6.1***	***18.9***	***49.7***	***858***	***12.2***	***27.0***
Beverages and Beverage Bases							
Energy, Sport, and Isotonic Drinks	13.8	0.8	na	10.0	142	8.4	17.3
Non-Milk Based Meal Replacement Beverages	0.8	<0.1*	na	0.5	11	9.0*	20.0*
Water (Bottled, Enhanced, Fortified)	85.5	5.2	16.7	44.4	793	11.7	25.1

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

Table A-3 Estimated Daily Intake of PQQ Disodium Salt from Individual Proposed Beverage-Uses by Female Teenagers Aged 12 to 19 Years Within the U.S. (2011-2012 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	All-Person Consumption (mg/day)		All-Users Consumption (mg/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***13.7***	***35.6***	***65.8***	***334***	***20.8***	***42.9***
Beverages and Beverage Bases							
Energy, Sport, and Isotonic Drinks	12.3	1.7	5.8*	12.8	59	13.2	20.3*
Non-Milk Based Meal Replacement Beverages	0	na	na	0	0	na	na
Water (Bottled, Enhanced, Fortified)	87.6	12.0	34.0	60.8	309	19.8	42.7

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

Table A-4 Estimated Daily Intake of PQQ Disodium Salt from Individual Proposed Beverage-Uses by Male Teenagers Aged 12 to 19 Years Within the U.S. (2011-2012 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	All-Person Consumption (mg/day)		All-Users Consumption (mg/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***14.8***	***41.5***	***60.1***	***314***	***24.7***	***58.3***
Beverages and Beverage Bases							
Energy, Sport, and Isotonic Drinks	19.6	2.9	10.4	24.5	94	11.8	22.3
Non-Milk Based Meal Replacement Beverages	1.6	0.2*	na	2.0	7	12.0*	14.8*
Water (Bottled, Enhanced, Fortified)	79.1	11.7	33.4	48.2	270	24.3	58.3

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

Table A-5 Estimated Daily Intake of PQQ Disodium Salt from Individual Proposed Beverage-Uses by Female Adults Aged 20 Years and Over Within the U.S. (2011-2012 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	All-Person Consumption (mg/day)		All-Users Consumption (mg/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***14.3***	***43.8***	***50.6***	***1,208***	***28.2***	***63.1***
Beverages and Beverage Bases							
Energy, Sport, and Isotonic Drinks	4.3	0.6	na	5.7	97	10.7	20.0
Non-Milk Based Meal Replacement Beverages	1.9	0.3	na	2.5	66	10.5	20.6*
Water (Bottled, Enhanced, Fortified)	93.7	13.4	42.9	45.8	1,134	29.3	66.2

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

Table A-6 Estimated Daily Intake of PQQ Disodium Salt from Individual Proposed Beverage-Uses by Male Adults Aged 20 Years and Over Within the U.S. (2011-2012 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	All-Person Consumption (mg/day)		All-Users Consumption (mg/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***15.4***	***52.2***	***47.0***	***1,091***	***32.7***	***69.7***
Beverages and Beverage Bases							
Energy, Sport, and Isotonic Drinks	12.4	1.9	6.0	12.2	220	15.6	29.5
Non-Milk Based Meal Replacement Beverages	2.9	0.4	na	2.8	63	15.9	31.0*
Water (Bottled, Enhanced, Fortified)	84.4	13.0	48.1	39.4	957	33.1	69.7

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

Table A-7 Estimated Daily Intake of PQQ Disodium Salt from Individual Proposed Beverage-Uses by the Total U.S. Population (2011-2012 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	All-Person Consumption (mg/day)		All-Users Consumption (mg/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***13.2***	***41.6***	***50.0***	***4,060***	***26.5***	***61.4***
Beverages and Beverage Bases							
Energy, Sport, and Isotonic Drinks	9.6	1.3	na	9.9	641	12.8	23.0
Non-Milk Based Meal Replacement Beverages	2.1	0.3	na	2.1	154	13.0	31.0
Water (Bottled, Enhanced, Fortified)	88.6	11.7	40.2	43.8	3,700	26.7	62.1

na = not available; NHANES = United States National Health and Nutrition Examination Survey; PQQ = pyrroloquinoline quinone; U.S. = United States.

Appendix B
Estimated Daily Per Kilogram Body Weight Intake of PQQ Disodium Salt from Individual Proposed Beverage-Uses by Different Population Groups Within the U.S.

Table B-1 Estimated Daily Per Kilogram Body Weight Intake of PQQ Disodium Salt from Individual Proposed Beverage-Uses by Infants and Young Children Aged 0 to 2 Years Within the U.S. (2011-2012 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	All-Person Consumption (mg/kg bw/day)		All-Users Consumption (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***0.2***	***0.8***	***33.4***	***253***	***0.7***	***1.5***
Beverages and Beverage Bases							
Energy, Sport, and Isotonic Drinks	9.8	<0.1*	na	3.7	29	0.6*	1.6*
Non-Milk Based Meal Replacement Beverages	3.4	<0.1*	na	1.2	7	0.7*	0.7*
Water (Bottled, Enhanced, Fortified)	87.1	0.2	0.7	31.1	236	0.6	1.5

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

Table B-2 Estimated Daily Per Kilogram Body Weight Intake of PQQ Disodium Salt from Individual Proposed Beverage-Uses by Children Aged 3 to 12 Years Within the U.S. (2011-2012 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	All-Person Consumption (mg/kg bw/day)		All-Users Consumption (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***0.2***	***0.7***	***49.7***	***858***	***0.4***	***1.0***
Beverages and Beverage Bases							
Energy, Sport, and Isotonic Drinks	12.6	<0.1	na	10.0	142	0.3	0.7
Non-Milk Based Meal Replacement Beverages	0.7	<0.1*	na	0.5	11	0.3*	0.7*
Water (Bottled, Enhanced, Fortified)	86.8	0.2	0.6	44.4	793	0.4	1.0

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

Table B-3 Estimated Daily Per Kilogram Body Weight Intake of PQQ Disodium Salt from Individual Proposed Beverage-Uses by Female Teenagers Aged 12 to 19 Years Within the U.S. (2011-2012 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	All-Person Consumption (mg/kg bw/day)		All-Users Consumption (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***0.2***	***0.6***	***66.0***	***326***	***0.3***	***0.7***
Beverages and Beverage Bases							
Energy, Sport, and Isotonic Drinks	11.2	<0.1	na	12.8	56	0.2	0.4*
Non-Milk Based Meal Replacement Beverages	0	na	na	0	0	Na	na
Water (Bottled, Enhanced, Fortified)	88.7	0.2	0.5	61.0	303	0.3	0.8

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

Table B-4 Estimated Daily Per Kilogram Body Weight Intake of PQQ Disodium Salt from Individual Proposed Beverage-Uses by Male Teenagers Aged 12 to 19 Years Within the U.S. (2011-2012 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	All-Person Consumption (mg/kg bw/day)		All-Users Consumption (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***0.2***	***0.6***	***60.1***	***313***	***0.4***	***0.8***
Beverages and Beverage Bases							
Energy, Sport, and Isotonic Drinks	20.9	<0.1	0.2	24.6	94	0.2	0.4
Non-Milk Based Meal Replacement Beverages	1.9	<0.1*	na	2.0	7	0.2*	0.3*
Water (Bottled, Enhanced, Fortified)	77.4	0.2	0.5	48.1	269	0.3	0.8

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

Table B-5 Estimated Daily Per Kilogram Body Weight Intake of PQQ Disodium Salt from Individual Proposed Beverage-Uses by Female Adults Aged 20 Years and Over Within the U.S. (2011-2012 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	All-Person Consumption (mg/kg bw/day)		All-Users Consumption (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***0.2***	***0.6***	***50.5***	***1,194***	***0.4***	***0.8***
Beverages and Beverage Bases							
Energy, Sport, and Isotonic Drinks	4.6	<0.1	na	5.6	95	0.2	0.3
Non-Milk Based Meal Replacement Beverages	1.9	<0.1	na	2.5	65	0.1	0.3*
Water (Bottled, Enhanced, Fortified)	93.3	0.2	0.6	45.7	1,120	0.4	0.9

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

Table B-6 Estimated Daily Per Kilogram Body Weight Intake of PQQ Disodium Salt from Individual Proposed Food-Uses by Male Adults Aged 20 Years and Over Within the U.S. (2011-2012 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	All-Person Consumption (mg/kg bw/day)		All-Users Consumption (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***0.2***	***0.6***	***46.9***	***1,079***	***0.4***	***0.9***
Beverages and Beverage Bases							
Energy, Sport, and Isotonic Drinks	12.8	<0.1	0.1	12.3	218	0.2	0.4
Non-Milk Based Meal Replacement Beverages	3.0	<0.1	na	2.8	63	0.2	0.4*
Water (Bottled, Enhanced, Fortified)	84.4	0.2	0.5	39.2	946	0.4	0.8

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

Table B-7 Estimated Daily Per Kilogram Body Weight Intake of PQQ Disodium Salt from Individual Proposed Food-Uses by the Total U.S. Population (2011-2012 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	All-Person Consumption (mg/kg bw/day)		All-Users Consumption (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***0.2***	***0.6***	***49.8***	***4,023***	***0.4***	***0.9***
Beverages and Beverage Bases							
Energy, Sport, and Isotonic Drinks	10.0	<0.1	na	9.9	634	0.2	0.4
Non-Milk Based Meal Replacement Beverages	2.0	<0.1	na	2.2	153	0.2	0.4
Water (Bottled, Enhanced, Fortified)	88.3	0.2	0.6	43.7	3,667	0.4	0.9

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

Appendix C
Representative NHANES Food Codes for Proposed Food-Uses of PQQ Disodium Salt in the U.S.

Representative NHANES Food Codes for Proposed Beverage-Uses of PQQ Disodium Salt in the U.S.

Beverages and Beverage Bases

Energy, Sport, and Isotonic Drinks
[PQQ Disodium Salt] = 0.0333 mg/g

95310200	Full Throttle Energy Drink
95310400	Monster Energy Drink
95310500	Mountain Dew AMP Energy Drink
95310550	No Fear Energy Drink
95310555	No Fear Motherload Energy Drink
95310560	NOS Energy Drink
95310600	Red Bull Energy Drink
95310700	Rockstar Energy Drink
95310750	SoBe Energize Energy Juice Drink
95310800	Vault Energy Drink
95311000	Energy Drink
95312400	Monster Energy Drink, Lo Carb
95312500	Mountain Dew AMP Energy Drink, sugar-free
95312550	No Fear Energy Drink, sugar-free
95312555	NOS Energy Drink, sugar-free
95312560	Ocean Spray Cran-Energy Cranberry Energy Juice Drink
95312600	Red Bull Energy Drink, sugar-free
95312700	Rockstar Energy Drink, sugar-free
95312800	Vault Zero Energy Drink
95312900	XS Energy Drink
95312905	XS Gold Plus Energy Drink
95320200	Gatorade Thirst Quencher sports drink
95320500	Powerade sports drink
95321000	Fruit-flavored thirst quencher beverage
95322200	Gatorade G2 Thirst Quencher sports drink, low calorie
95322500	Powerade Zero sports drink, low calorie
95323000	Fruit-flavored sports drink or thirst quencher beverage, low calorie
95330100	Fluid replacement, electrolyte solution
95330500	Fluid replacement, 5% glucose in water

Non-Reconstituted Sport Drinks
Adjusted for not being reconstituted, 16 g of powder to 240 mL of water
[PQQ Disodium Salt] = 0.53 mg/g

92900300	Fruit-flavored thirst quencher beverage, dry concentrate, not reconstituted

Non-Milk Based Meal Replacement Beverages
[PQQ Disodium Salt] = 0.0333 mg/g

95101000	Boost, nutritional drink, ready-to-drink
95101010	Boost Plus, nutritional drink, ready-to-drink
95104000	Glucerna, nutritional shake, ready-to-drink
95105000	Kellogg's Special K Protein Shake
95110000	Slim Fast Shake, meal replacement, regular, ready-to-drink
95110010	Slim Fast Shake, meal replacement, sugar free, ready-to-drink

95110020	Slim Fast Shake, meal replacement, high protein, ready-to-drink
95120000	Nutritional drink or meal replacement, ready-to-drink, NFS
95120010	Nutritional drink or meal replacement, high protein, ready-to-drink, NFS
95120020	Nutritional drink or meal replacement, high protein, light, ready-to-drink, NFS
95120050	Nutritional drink or meal replacement, liquid, soy-based

Non-Reconstituted Non-Milk Based Meal Replacement Beverages
Adjusted for not being reconstituted, 16 g of powder to 240 mL of water
[PQQ Disodium Salt] = 0.53 mg/g

95201300	EAS Soy Protein Powder
95201500	Herbalife, nutritional shake mix, high protein, powder
95201600	Isopure protein powder
95201700	Kellogg's Special K20 Protein Water Mix
95210000	Slim Fast Shake Mix, powder
95210010	Slim Fast Shake Mix, sugar free, powder
95210020	Slim Fast Shake Mix, high protein, powder
95220000	Nutritional drink mix or meal replacement, powder, NFS
95220010	Nutritional drink mix or meal replacement, high protein, powder, NFS
95230010	Protein powder, soy based, NFS
95230020	Protein powder, light, NFS
95230030	Protein powder, NFS

Water (Bottled, Enhanced, Fortified)
[PQQ Disodium Salt] = 0.033 mg/g

94100100	Water, bottled, unsweetened
94100200	Water, bottled, sweetened, with low or no calorie sweetener
94100300	Water, fruit flavored, sweetened, with high fructose corn syrup and low calorie sweetener
94210100	Propel Water
94210200	Glaceau Water
94210300	SoBe Lifewater
94220200	Glaceau Water, low calorie

SUBMISSION END