View Notification

NDI Number	Filing Date	Category of Compound
2017001013	03/21/2017	

Section 1: Contact Information

Contacts List

Type	Name	Address
Submitter	(b) (4), (b) (6)	
Owner	NutraLand	212 Technology Dr Ste X, Irvine, CA 92618-2419 United States
Primary	(b) (4), (b) (6)	

Submitter

Type of Submitter
Agent / Attorney / Consultant

Company Name *(if applicable)*
(b) (4), (b) (6)

Owner

Type of Submitter
Distributor of NDI

Company Name *(if applicable)*
NutraLand

Corrected Company Name

Mailing Address Line 1
212 Technology Dr Ste X

Mailing Address Line 2

Country
United States

ZIP or Postal Code
92618-2419

City
Irvine

State or Province
CA

Primary

Type of Contact
Agent / Attorney / Consultant

First Name of Contact Person
(b) (4), (b) (6)

Mailing Address
(b) (4), (b) (6)

Section 2: General Administrative Information

1. Name of the New Dietary Ingredient
Pyrroloquinoline Quinone (PQQ) Disodium Salt

2. Have you designated informa ion in your notifica ion that you view as a trade secret or as confiden ial commercial information?
Yes, information is designated at the place where it occurs in the notification

3. Are you providing a redacted copy of some or all of the notification?
Yes, redacted copy of part(s) of the notification

4. Are all citations to published information accompanied by reprints or full photostatic copies of the publications?
Yes

5. Are the notification and all publica ions submitted in English or accompanied by a complete and accurate English translation?
Yes

Section 3: Description of NDI and Dietary Supplement Containing the NDI

1. New Dietary Ingredient Type
Vitamin

Dietary substance for use by man to supplement the diet by increasing the total dietary intake

2. Name of the new dietary ingredient and related information
Maximum level of new dietary ingredient in each serving of dietary supplement (include units)

30 mg per serving per day

NDI Name
Pyrroloquinoline Quinone (PQQ) Disodium Salt

Corrected NDI Name

Synonyms and Trade Name
PQQ

Corrected Synonyms and Trade Name

Plant Part and Strain

Latin Binomial Name (LBN)

Correcetd Latin Binomial Name (LBN)

Au hor of LBN

Corrected Author of LBN

3. Dietary supplement serving form
Powder

4. Description of dietary supplement (Include the level of NDI and all o her ingredients in one unit of the dietary supplement. If the notification concerns an NDI that is a combination of two or more other NDIs, you should provide the following information for each component NDI: Synonyms, Trade Name, Plant Part, Strain, Latin Binomial Name, Author of Latin Binomial Name, and NDI type. Where relevant, also include the following additional informa ion: CAS registry number, Unusual form (e.g., malted barley or immature apples), Type of manufacture (e.g., greater than 99% purity, 50:1 dry leaf extract, or fermentation product)).
Synonym: Methoxatin disodium salt, Disodium pyrroloquinolinedione tricarboxylate CAS Registry Number: 122628-50-6 Molecular Formula: C14H4N2Na2O8; Molecular Weight: 374.17; Purity-99% or higher Recommended daily dose is up to 30 mg per person per day, which is only one half of the dose described in NDIN RPT 417.

5. Conditions of Use of the Dietary Supplement

5a. Serving instructions (e.g., "take with food", "take before bed", "dissolve in a glass of water", etc).
Can be consumed in tablet or capsule form or can be incorporated into a drink form.

5b. Dietary supplement serving size (weight or volumetric measure), serving frequency (# of servings/day, interval between servings), duration of use and maximum total daily intake level
30 mg per serving, once a day; or 15 mg/per serving, twice a day

5c. Target populations / excluded populations / other restrictions
Adults aged 18 years or older

Section 4: Safety Information Attachment

Attachment

Name of Attachment	Size (KB)	Date of Upload
PQQ NDI 3-21-2017 final.doc	2859.0	03/21/2017

Section 5: Additional Attachments

Attachment(s)

Name of Attachment	Size (KB)	Date of Upload
Ref 2. CDC 2009.pdf	29.52	03/21/2017
Ref 18. Rucker 2009 PQQ review.pdf	505.4	03/21/2017
Ref 11. Nakano 2016.pdf	335.96	03/21/2017
Ref 21. Urakami 1992.pdf	1280.44	03/21/2017
Ref 9. Kumazawa 1995 PQQ levels in foods.pdf	388.44	03/21/2017
Ref 3. FDA 2007. NDI 0417 PQQ orig submission 2007.pdf	1379.28	03/21/2017
Ref 17. Nakano 2009 PQQ mental status (1).pdf	239.88	03/21/2017
Ref 5. GRN 625.pdf	5263.96	03/21/2017
Ref 7. Itoh 2016.pdf	160.78	03/21/2017
Ref 8. Kano 1990 PQQ dissociation.pdf	928.96	03/21/2017
Ref 6. Harris 2013 PQQanti-inflamm human.pdf	1420.33	03/21/2017
Ref 14. Nakano 2014 PQQ acute-subchronic tox in rats.pdf	600.42	03/21/2017
Ref 4. GRN 641 Hisun's PQQ.pdf	1019.7	03/21/2017
Ref 12. Nakano 2015a human.pdf	651.41	03/21/2017
Ref 1. ATCC 2017.pdf	38.66	03/21/2017
Ref 10. Liang2015 PQQ subchronic tox in rats.pdf	299.36	03/21/2017
Ref 13. Nakano 2015b PQQ healthy women skin.pdf	622.47	03/21/2017
Ref 19. Smidt 1991 PQQ metabolism.pdf	433.53	03/21/2017
Ref 16. Nakano 2012 Func Food Health Dis.pdf	666.03	03/21/2017
Ref 20. Steinberg 2003 PQQ mice.pdf	7743.48	03/21/2017
Ref 15. Nakano 2013 PQQ genotoxicity.pdf	348.97	03/21/2017

Section 6: Certification

Name of Submitter
(b) (4), (b) (6)

Title of Submitter
Presdent

I certify that the information in he notification is true and accurate and that I am authorized to submit the notification on behalf of he notification owner.

☑ **I Agree.**

NEW DIETARY INGREDIENT NOTICE OF PYRROLOQUINOLINE QUINONE DISODIUM SALT

Prepared for: Nutraland and (b) (4)

Prepared by: (b) (4), (b) (6)
(b) (4), (b) (6)

NDI notice

Table of Contents

Tables and Figures

Section 1. New Dietary Ingredient (NDI) Identity Information

1.1 Identity of the NDI

Chemical Name: Pyrroloquinoline Quinone Disodium Salt
Synonyms: Methoxatin disodium salt, Disodium pyrroloquinolinedione tricarboxylate
Chemical Abstracts Service (CAS) Number: 122628-50-6
Molecular Formula: $C_{14}H_4N_2Na_2O_8$
Molecular Weight: 374.17
Melting Point: > 300 °C
Solubility: 3 g/L (25 °C) in water; insoluble in organic solvents

Distributor: (b) (4)
Responsible person: Dr. Lin Ling
212 Technology Dr., Suite X, Irvine, CA 92618, USA
E mail: xu.sanying@nutralandusa.com
Phone: (949) 988 7615 Fax: (949) 988 7616
Manufacturer: (b) (4)

Background:

Pyrroloquinoline quinone (PQQ) is a water-soluble quinone compound, first identified in 1979, from bacteria as a cofactor for redox enzymes (Itoh et al., 2016). It is contained in various foods and beverages such as parsley, green tea, or fermented soybeans. PQQ is also detected in various human organs and tissues, and is especially high in human breast milk. PQQ has anti-oxidative and mitochondrial biogenesis capacities (Itoh et al., 2016).

The importance of PQQ in the health of mammals is shown when it is omitted from chemically defined diets (Rucker et al., 2009). PQQ deficiency results in a wide variety of systemic responses, including compromised immune function, growth impairment, and abnormal reproductive performance in mice and rats (Steinberg et al., 2003). Improvements in mitochondrial respiratory function are potentially important to a variety of health issues, including energy utilization and protection from reactive oxygen species. PQQ has also been found to attenuate neuronal cell death in stroke and spinal cord injury models and protect against cardiac damage from ischemia. In addition, it has been demonstrated that PQQ is potentially effective for preventing neurodegeneration caused by oxidative stress, and may improve memory (Itoh et al., 2016). These factors suggest PQQ plays an important role in human health. PQQ is present in many plant foods, such as parsley, green peppers, kiwi, papaya, and tofu (Kumazawa et al., 1995). Figure 1 shows chemical structure of PQQ.

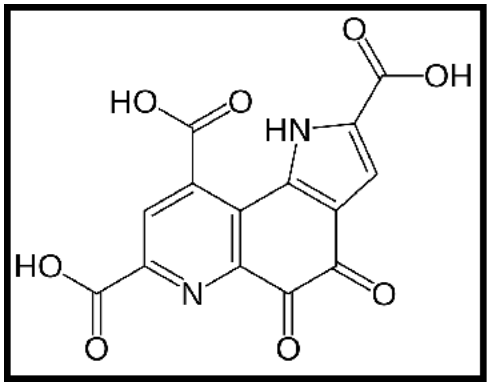


Figure 1. Chemical Structure of PQQ

1.2 Description of the evidence verifying the identity of the NDI

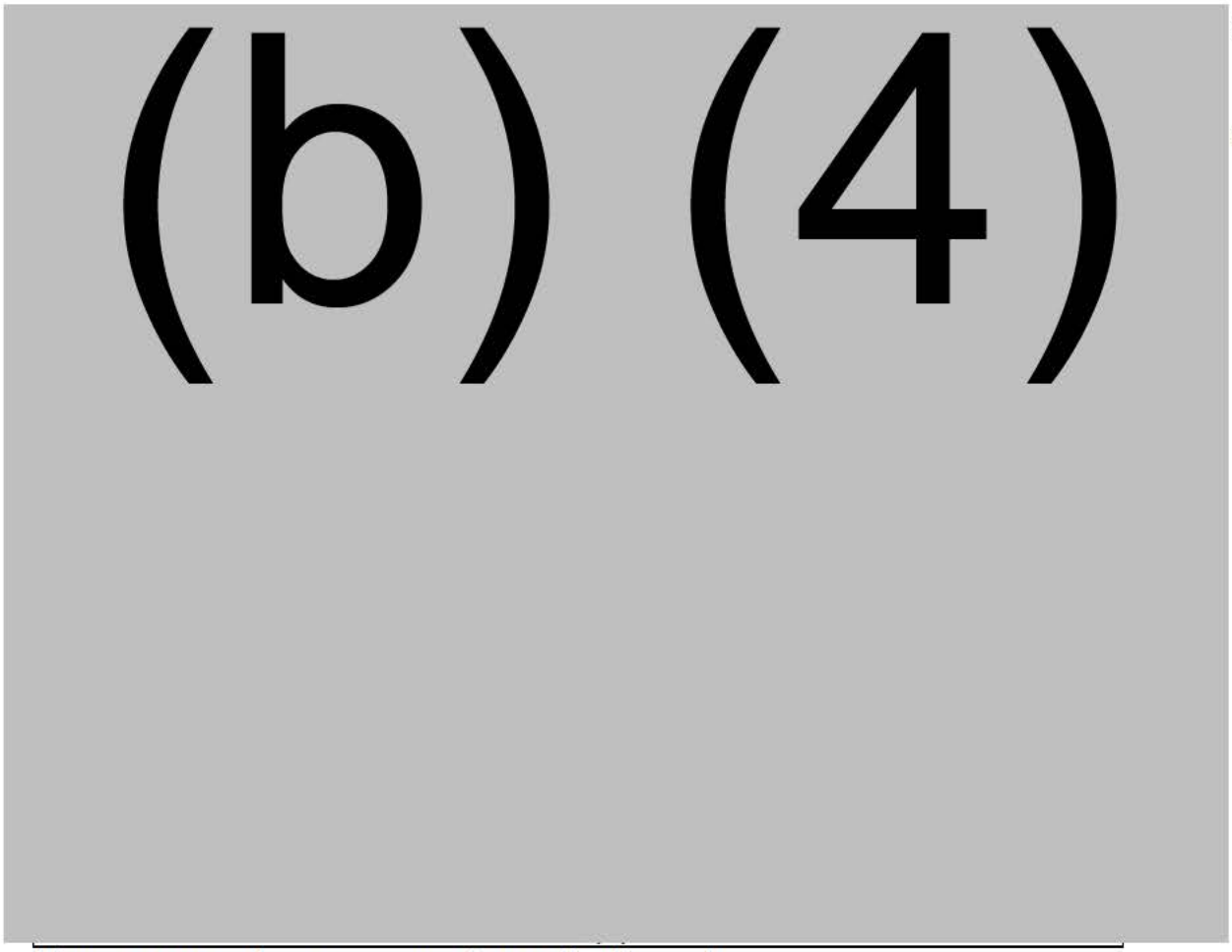


Figure 2. HPLC chromatograms of PQQ disodium salt

Other Identification Methods

In addition to HPLC chromatograms, NMR, UV absorption and FT-IR spectra confirmed that the notified substance is PQQ disodium salt. Details are presented in Appendix B.

1.3 NDI manufacture

The entire Section 1.3 is confidential (a bottom part of page 5-page 9)

1.3.1 Raw materials

Table 1 lists raw materials used in the manufacture of PQQ disodium salt.

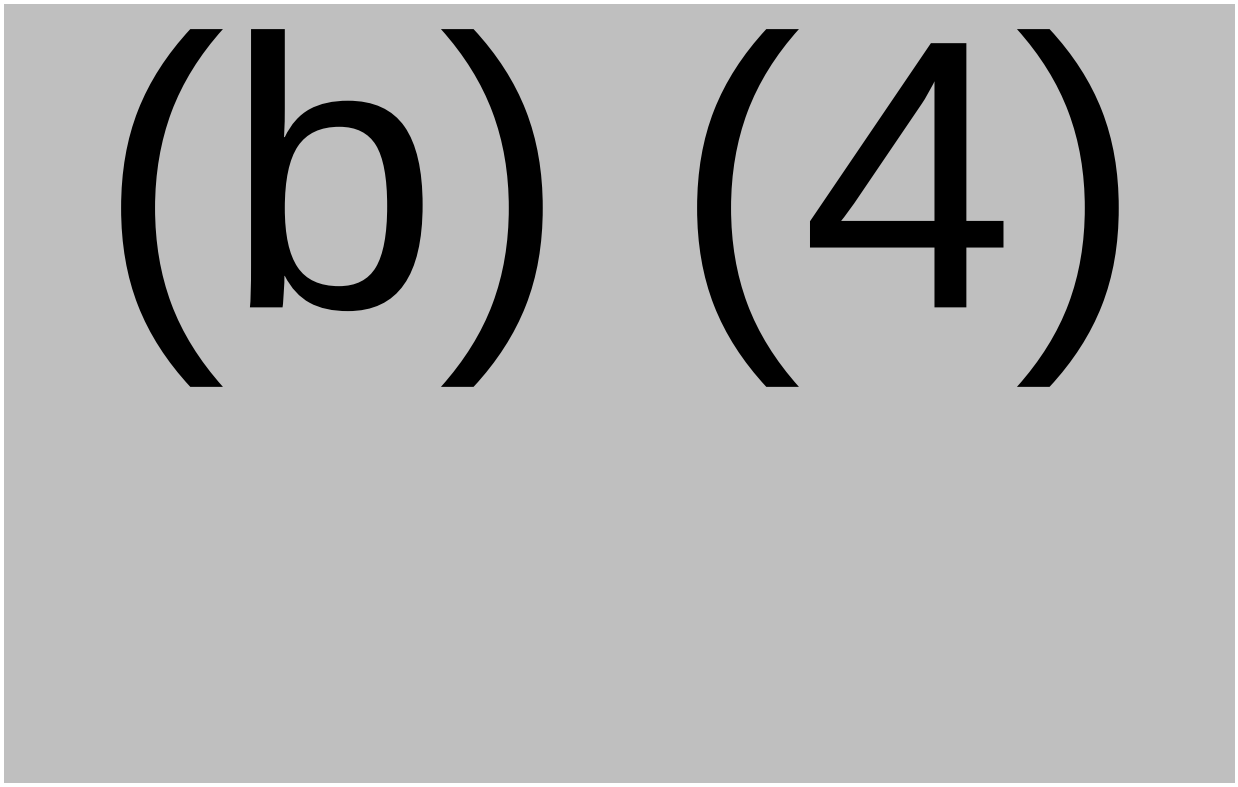
Table 1. Raw materials used in manufacture of NutraLand's PQQ disodium salt

(b) (4)

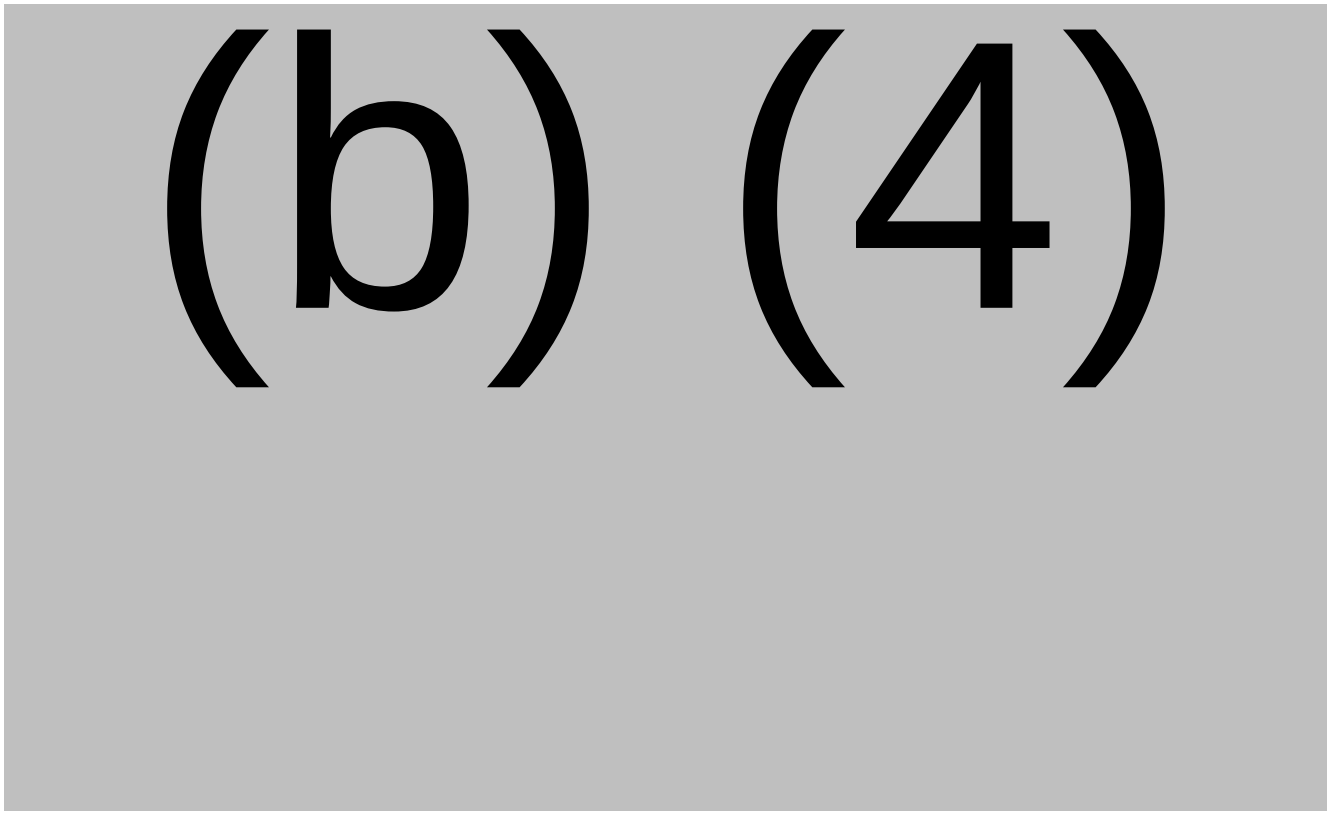
Specifications and certificate of analysis (COA) of raw materials is found in Appendix C.

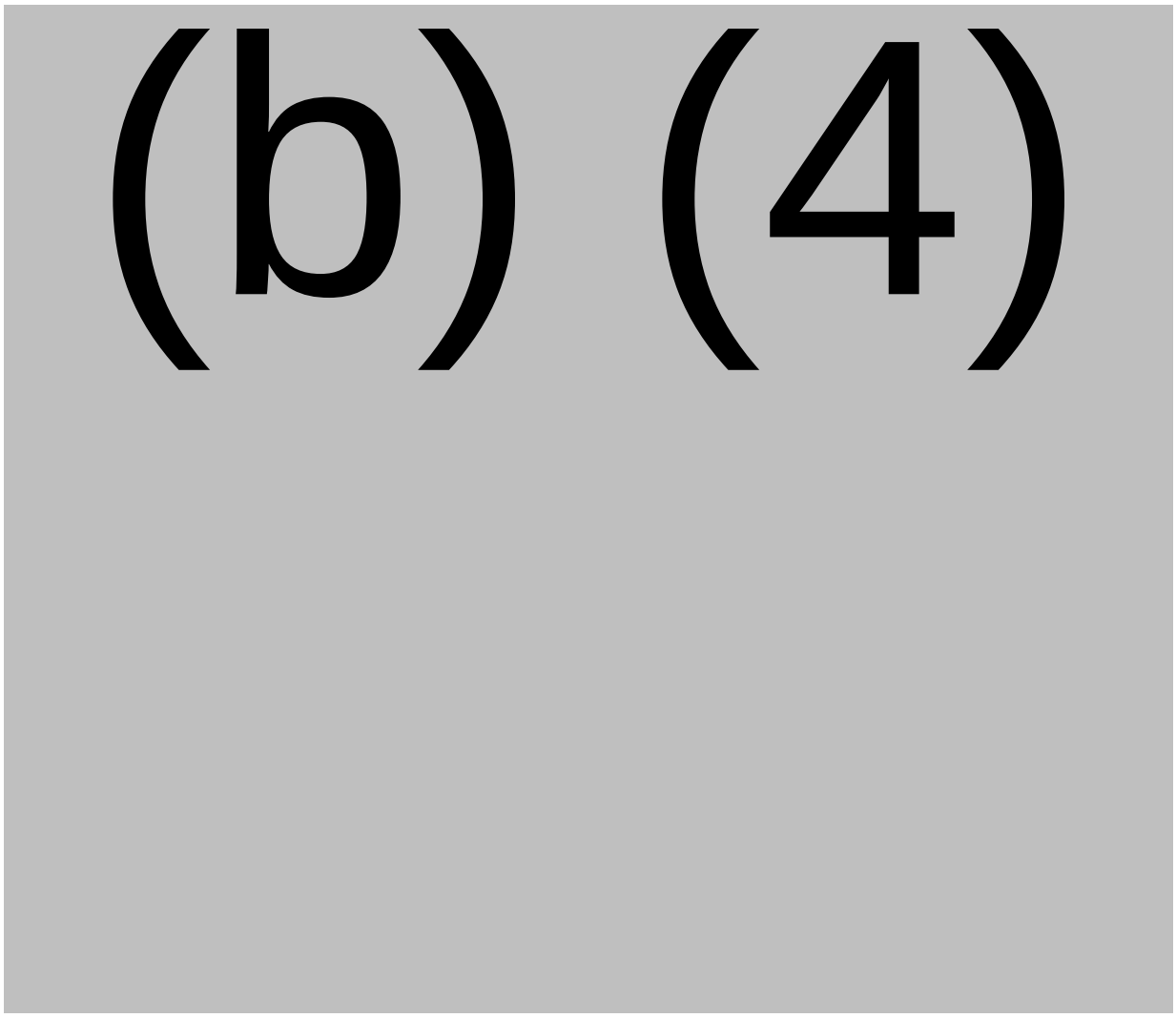
1.3.2 Formulation ingredients

(b) (4)



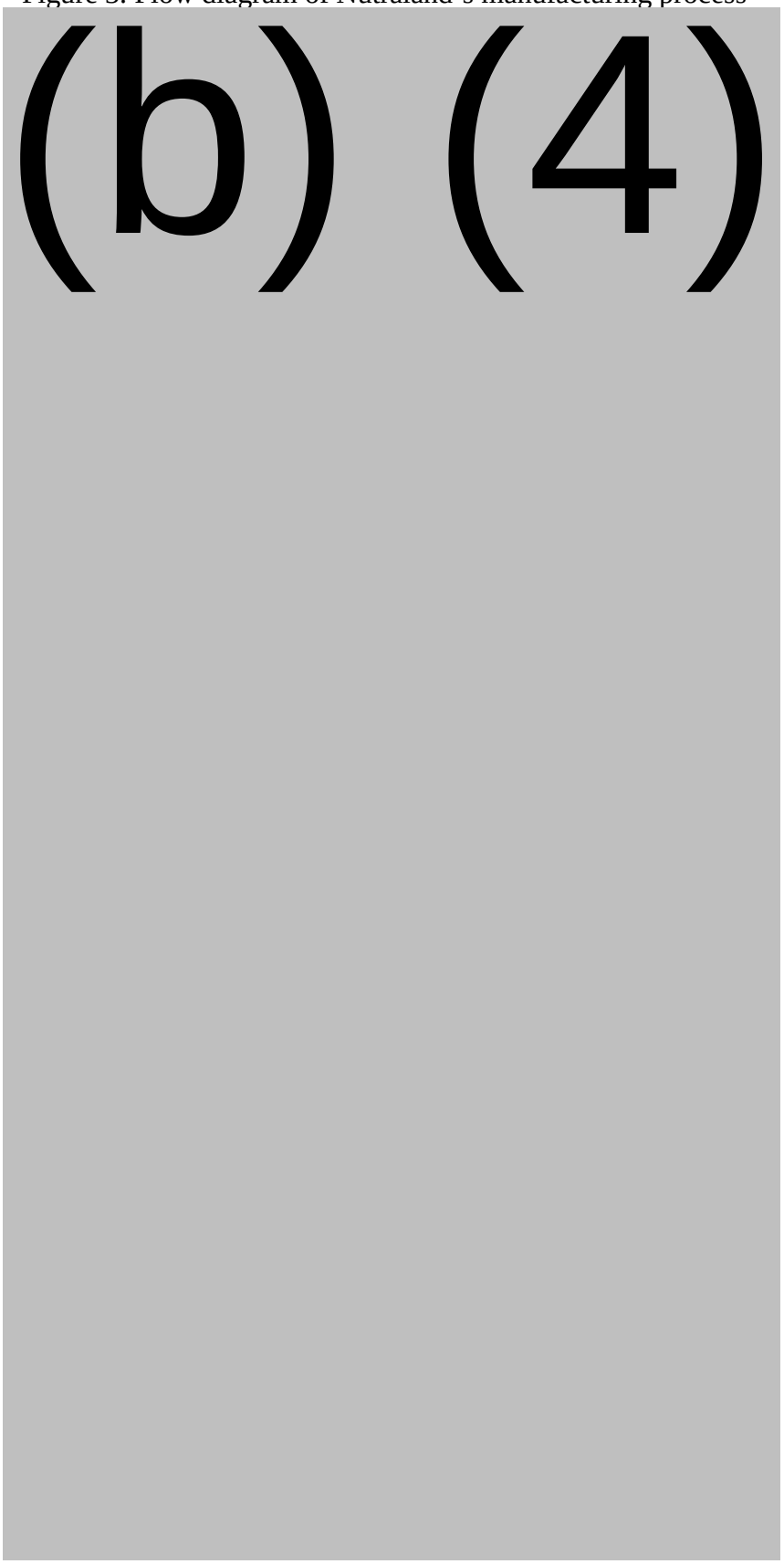
1.3.3 Manufacturing process





(b) (4)

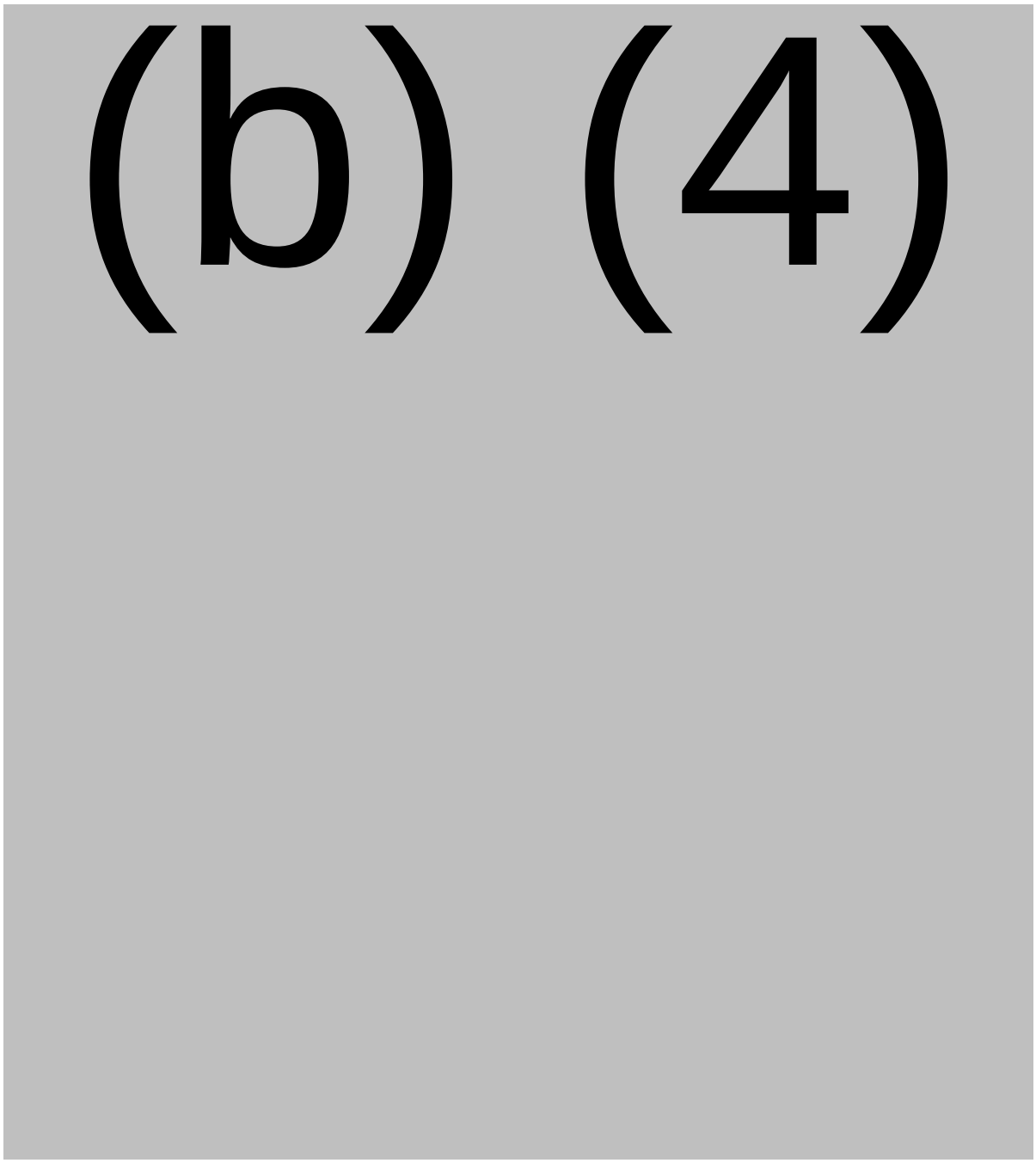
Figure 3. Flow diagram of Nutraland's manufacturing process



1.3.4 NDI specifications

Table 3 shows the specifications of PQQ disodium salt.

Table 3. Specifications of PQQ disodium salt



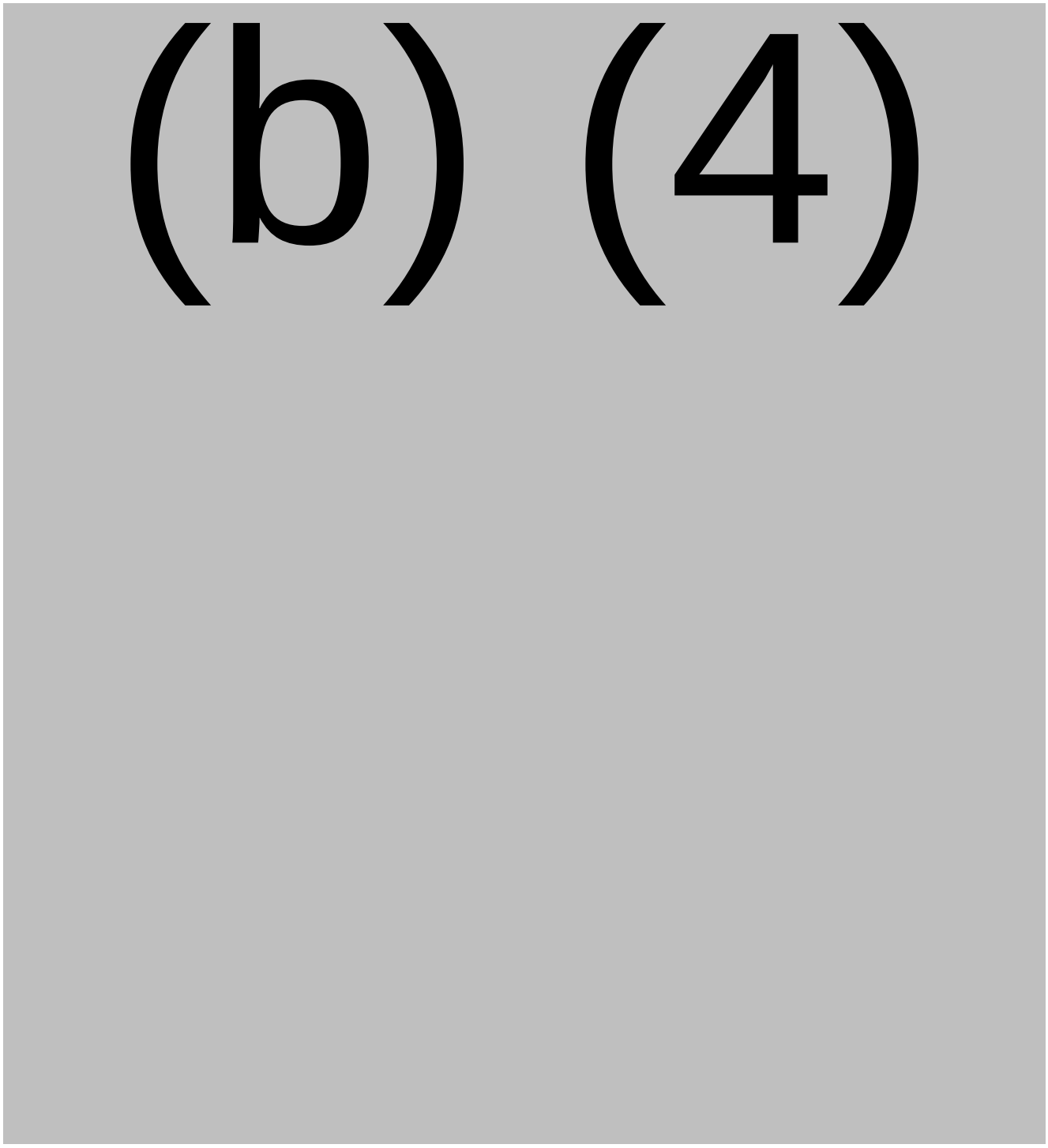
(b) (4)

1.3.5 Methods of analysis

The method of analysis is included in Table 3 in Section 1.3.4.

1.3.6 Safety of bacteria used in fermentation

(b) (4)


(b) (4)

(b) (4)

1.3.7 Disintegration and dissolution profile

(b) (4)

1.3.8 Shelf-life

RPT 417 reported that PQQ disodium salt was stable at 50 degrees centigrade in the dark and at 30 degrees centigrade under light conditions (1575 Ix, LTV 26μW/cm) for more than 12 months. Stability tests showed that PQQ disodium salt is stable for at least 12 months unaffected by heat (up to 50 degrees centigrade), humidity (Relative humidity, 75%) and light.

References for Section 1.

1. ATCC. 2016. https://www.atcc.org/Products/All/51888.aspx.

2. CDC, 2009. Section IV- Laboratory Biosafety Level Criteria, *Biosafety in Microbiological and Biomedical Laboratories,* eds. L.C. Chosewood and D.E. Wilson. Atlanta, GA: Centers for Disease Control and Prevention, accessed online at http://www.cdc.gov/biosafety/publications/bmb15/BMBL5 sect IV.pdf.

3. Food and Drug Administration (FDA). 2007 RPT 417. A NDI notice for pyrroloquinoline quinone (PQQ) disodium salt as dietary ingredient for dietary supplements, filed by Mitsubishi Gas Chemical Co., Inc.

4. Food and Drug Administration (FDA). 2016. GRN 641. A GRAS notice for pyrroloquinoline quinone (PQQ) disodium salt, filed by Zeijang Hisun Pharmaceutical Co, Ltd.

5. Itoh Y, Hine K, Miura H, Uetake T, Nakano M, Takemura N, Sakatani K. Effect of the Antioxidant Supplement Pyrroloquinoline Quinone Disodium Salt (BioPQQ™) on Cognitive Functions. Adv Exp Med Biol. 2016;876:319-25.

6. Kano K, Mori K, Uno B, Kubota T. Voltametric determination of acid dissociation constants of pyrroloquinoline quinone and its reduced form under acidic conditions . Bioelectro Bioenerg. 1990;24:193-201.

7. Kumazawa T, Sato K, Seno H, Ishii A, Suzuki O. Levels of pyrroloquinoline quinone in various foods. Biochem J. 1995; 307(2):331-333.

8. Rucker R, Chowanadisai W, Nakano M. Potential physiological importance of pyrroloquinoline quinone. Altern Med Rev.2009;14:268–77.

9. Steinberg F, Stites TE, Anderson P, Storms D, Chan I, Eghbali S, Rucker R. Pyrroquinoline quinone improves growth and reproductice performance in mice fed chemically defined diets. Exp Biol Med. 2003;228:160-6.

10. Urakami T, Yashima K, Kobayashi H, Yoshida A, Ito-Yoshida C (1992). Production of pyrroloquinoline quinone by using methanol-utilizing bacteria. Appl Environ Microbiol 58(12):3970-3976.

Section 2. Dietary Supplement Manufacture

Not applicable.

Section 3. History of Use or Other Evidence of Safety

3.1 History of use

3.1.1 Description of the relationship between the historically consumed material and the NDI or dietary supplement containing the NDI

In the past decade, PQQ has been safely used as dietary supplements at daily doses up to 60 mg per day. Mitsubishi Gas Company has estimated that daily consumption of PQQ from foods ranged from 0.01 to 0.4 mg per day (RPT 417).

3.1.2 Describe identity information verifying the relationship between the historically consumed material and the NDI or dietary supplement containing the NDI

Nutraland intends to use PQQ disodium salt as a bulk ingredient of dietary supplement. Recommended daily dose is 30 mg per person per day, which is 3 times lower than the dose described in NDIN RPT 417.

3.1.3 Historical conditions of use and cumulative exposure estimate for the historically consumed material

In the past decade, PQQ has been safely used as dietary supplements at daily doses up to 60 mg per day.

3.1.4 Adverse events associated with historically consumed material

Since the introduction of PQQ in the early 2000s, no significant adverse events have been reported.

3.1.5 Alternative rationale for reasonable expectation of safety based on history of use

Not applicable.

3.2 Other evidence of safety

The FDA has issued a 'no objection' letter on a NDI notice related to PQQ produced by a (b) (4) (RPT 417 filed by Mitsubishi Gas Chemical Co., Inc.). As the PQQ disodium salt in this NDI notice is similar in specifications compared to the PQQ disodium salt in the previous FDA NDI notice (filed by Mitsubishi), it is recognized that the information and data in NDIN RPT 417 are pertinent to the safety of the PQQ disodium salt in this NDI notice. Therefore, this notice incorporates, by reference, the safety and metabolism studies discussed in the previous NDI notice. Additionally, this notice discusses an additional animal study that has been published since the FDA's last review. The subject of the present NDI notice is PQQ disodium salt produced via (b) (4)). It is noteworthy that the intended use level in this notice (30 mg/person/day) is much lower than the level described in NDIN RPT 417.

Toxicity studies conducted with PQQ disodium salt manufactured by Nutraland are limited to evaluations of acute toxicity in rats, mutagenicity and genotoxicity and sperm health in mice. However, the acute and subchronic oral toxicity study results (up to 90 days) of another source of high purity PQQ disodium salt (BioPQQ; Mitsubishi Gas Chemical Co., Inc.; produced by *Hyphomicrobium denitrificans* fermentation) were presented in RPT 417 (FDA, 2007) and later published by Nakano *et al*. (2013). In addition, the safety data of PQQ disodium salt of similar purity (manufactured by Hisun using a similar production process, including fermentation by *Hyphomicrobium denitrificans*) are also available in GRN 641. The results of these studies were considered applicable to the safety assessment of Nutraland's PQQ disodium salt ingredient on the basis of similarities in manufacturing process, composition and specifications (Table 6).

In addition, high purity PQQ disodium salt ingredients manufactured by (b) (4) . have also been the subject of toxicity testing (Liang et al., 2015; Table 6). Although details of the manufacturing processes of the PQQ disodium salt ingredients used in these studies have not been identified, these studies were evaluated as corroborative safety studies due to similarity in specifications. Indeed, LD50 values of NutraLand's PQQ disodium salt are comparable to those described in other NDIN (RPT 417) and GRAS notices (GRN 625 and 641). Details of toxicity studies are presented in Sections 3.2.2 and 3.2.3.

Table 6. Comparison of the PQQ disodium salt preparations used in the animal studies

Parameter	Source				
	NutraLand	Hisun	Mitsubishi	Shanghai Med Co	Nascent Health
Regulatory status	Current notice	GRN 641	NDIN RPT 417	NA	GRN 625
Manufacturing Method	(b) (4)				
Appearance	Reddish brown crystalline powder	Henna powder	Reddish brown crystalline powder	Reddish brown crystalline powder	Reddish brown crystalline powder
LD_{50}, g/kg bw	(b) (4)				
NOAEL from a 90-day rat oral toxicity study	NA	NA	100 mg/kg bw/day	400 mg/kg bw/day	400 mg/kg bw/day
Purity	>99%	>99%	>99%	>98%	>98%

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

3.2.1. Metabolism

Since the FDA's last review of RPT 417 in 2007, one human metabolism study has been published assessing PQQ intake over the course of two time periods (Table 7).

Harris et al. (2013) performed a crossover assessment with 10 subjects (5 females, 5 males), who ingested PQQ (source; not specified) added to a fruit-flavored drink in two separate studies, reporting that serum concentrations of PQQ in humans increased directly in response to an increase in dietary intake, and daily urinary excretion of PQQ was related to both serum levels and daily intake ($r=0.9$, $p<0.05$).

In the first of the two studies conducted by Harris et al. (2013), a single dose of PQQ was administered (0.2 mg PQQ/kg body weight [bw]). In the following 48 hours, measurements of plasma and urine PQQ levels as well as changes in antioxidant potential were taken. Standard clinical indices were normal and not altered by PQQ supplementation. Dietary PQQ exposure resulted in slight changes in antioxidant potential based on malonaldehyde-related TBAR assessments (12% decrease in reactive oxidant products, $p<0.01$).

In the second study by Harris et al. (2013), a daily dose of PQQ (source, not specified) was administered (0.3 mg PQQ/kg bw/day). After 76 h, measurements included indices of inflammation (plasma C-reactive protein [CRP], interleukin [IL]-6 levels), standard serum clinical indices (e.g., total cholesterol [TC], glucose, high density lipoprotein cholesterol [HDL-C], low-density lipoprotein cholesterol [LDL-C], triglycerides [TG], creatine, uric acid, total protein, aspartate transaminase) and 1H-nuclear magnetic resonance (NMR) estimates of urinary metabolites related in part to oxidative metabolism. The standard clinical indices were normal and not altered by PQQ supplementation. Standard clinical indices were normal and not altered by PQQ supplementation. PQQ supplementation resulted in significant decreases in the levels of plasma CRP (by approximately 55%), IL-6 (by approximately by 35%), and urinary concentrations of total amino acids (by approximately 15%) and methylated amines. It also caused changes in urinary metabolites consistent with enhanced mitochondria-related functions.

Table 7. Summary of human metabolism studies published since the FDA's 2007 review

Subjects	Dose	Duration	Measured Outcome	Results	Author
Studies published since the 2007 FDA's review of PQQ					
10 male and female subjects, 21-34 y	0.2 mg/kg bw	48 h	Plasma/urine PQQ levels, antioxidant potential, standard clinical indices	PQQ slightly increased antioxidant potential	Harris et al., 2013
10 male and female subjects, 21-34 y	0.3 mg/kg bw/d	76 h	Inflammation indices, serum clinical indices, and estimates of urinary metabolites	PQQ significantly decreased plasma levels of CRP (by ~55%) and IL-6 (by ~ 35%) and urinary concentrations of total amino acids (by ~15%). The data indicated increased mitochondria-related functions	Harris et al., 2013

Abbreviations: bw=body weight; CRP= C-reactive protein; FDA= Food and Drug Administration; h= hour; PQQ= pyrroloquinoline quinone; y=years.

The RPT 417 included the study by Smidt et al. (1991). In this study, 1.5 mg/kg PQQ (labeled with ^{14}C) was administered to mice orally, approximately 62% of the PQQ was absorbed through the small intestine and 81% of that absorbed PQQ was excreted within 24 hours via the kidneys (Table 4). The radioactive PQQ was detected in the kidney (10.7%) and the skin (1.3%) 24 hours after oral administration. This shows PQQ is absorbed effectively, but most of the PQQ absorbed is excreted into the urine.

Reference in the Section 3.2.1.

1. M,Harris CB, Chowanadisai W, Mishchuk DO, Satre MA, Slupsky CM, Rucker RB. Dietary pyrroquinoline quinone (PQQ) alters indicators of inflammation and mitochondrial-related metabolism in human subjects. J Nutr Biochem. 2013;24:2076-84.

2. Smidt CR, Unkefer CJ, Houck DR, Rucker RB. Intestinal absorption and tissue distribution of [14C]pyrroloquinoline quinone in mice. Proc Soc Exp Biol Med. 1991;197:27-31.

3.2.2. Mutagenicity and genotoxicity studies

Mutagenicity and genotoxicity studies of PQQ disodium salt are summarized in Table 8.

3.2.2.1. Studies on Nutraland's PQQ disodium salt

(b) (4)

(b) (4)

3.2.2.2. Studies on Other Sources of PQQ disodium salt (Studies included in FDA's previous reviews)

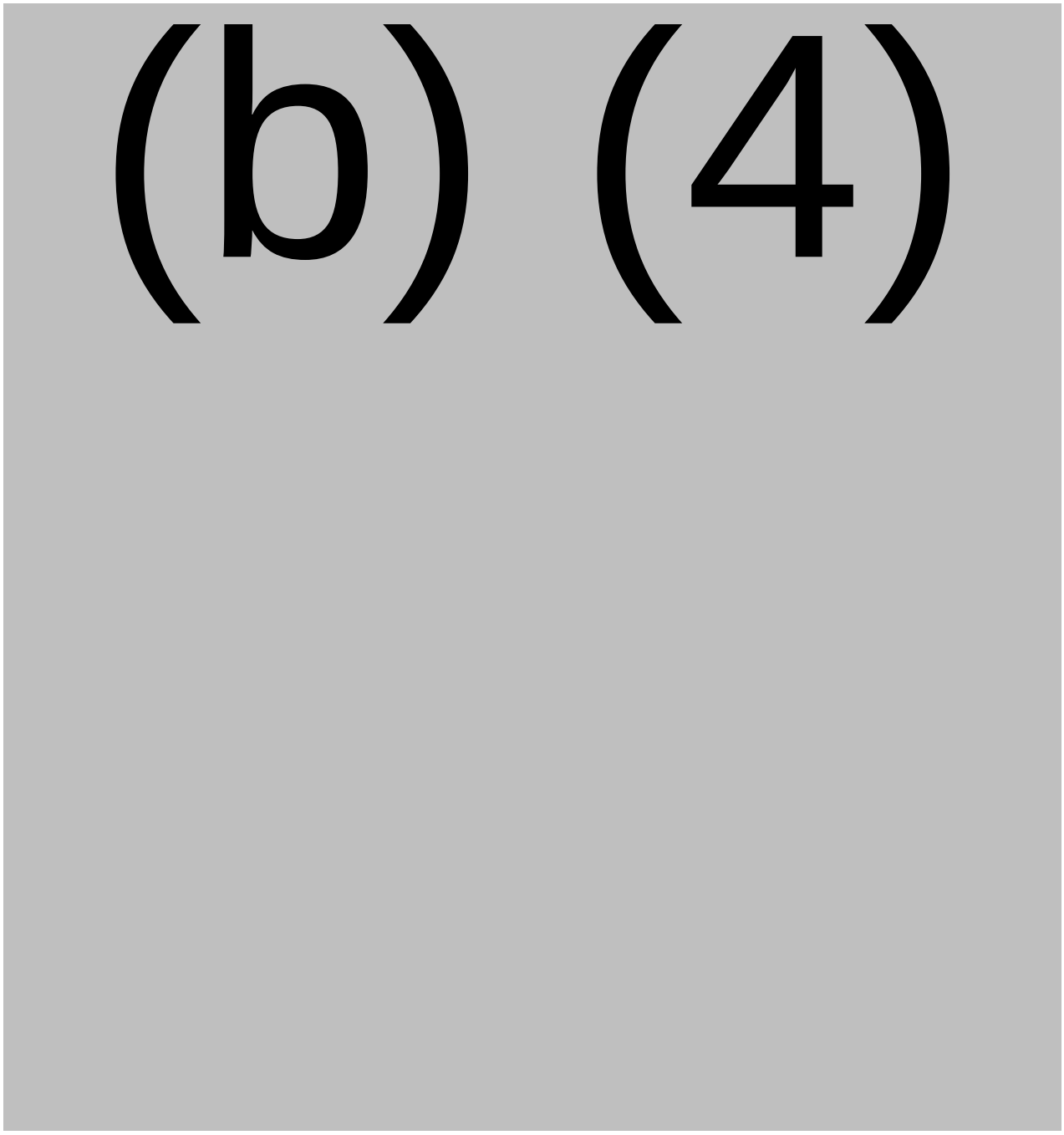
Since the FDA's last review of NDIN RPT 417 in 2007, no new studies have been published assessing other sources of PQQ. However, Zeijang Hisun Pharmaceutical Co., Ltd. (Hereinafter called as "Hisun"), reported in its GRAS filing (GRN 641) that its PQQ disodium salt obtained from microbial fermentation by *Hyphomicrobium denitrificans* was not mutagenic or genotoxic.

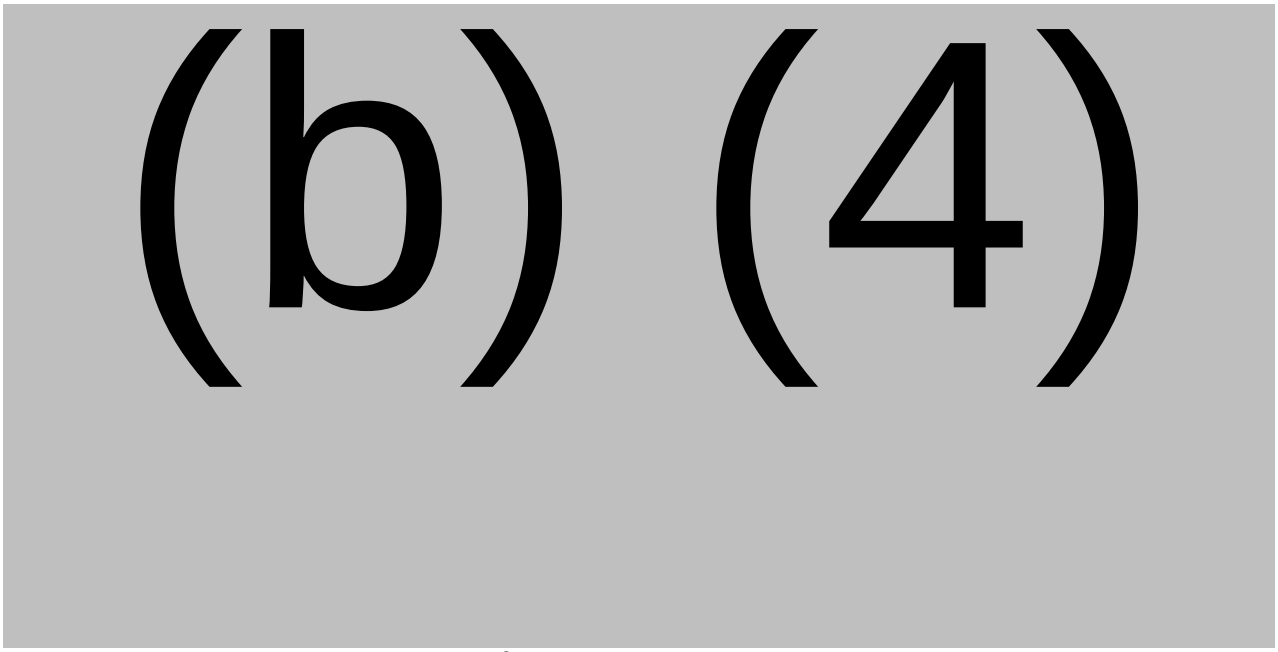
RPT 417 (FDA, 2007) reported a series of mutagenicity and genotoxicity studies (Ames test, chromosomal aberration tests, and *in vivo* mouse micronucleus assay). These studies were later published by Nakano et al. in 2013. The studies of genotoxic potential of PQQ disodium salt are summarized in Table 9. Manufacturing processes of the PQQ disodium salt used in these studies (b) (4) Thus, the results of these

studies can be used in evaluating the mutagenic and/or genotoxic potential of Nutraland's PQQ disodium salt.

Additionally, synthetically manufactured PQQ disodium salt also showed that it lacked mutagenicity and genotoxicity (FDA, 2016b). Overall, studies consistently show that all preparations of PQQ disodium salt are not mutagenic or genotoxic.

Table 8. Mutagenicity and genotoxicity studies of PQQ disodium salt





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

Reference for the Section 3.2.2.

1. Food and Drug Administration (FDA). 2007 RPT 417. A NDI notice for pyrroloquinoline quinone (PQQ) disodium salt as dietary ingredient for dietary supplements, filed by Mitsubishi Gas Chemical Co., Inc.

2. Food and Drug Administration (FDA). 2016. GRN 641. A GRAS notice for pyrroloquinoline quinone (PQQ) disodium salt, filed by Zeijang Hisun Pharmaceutical Co, Ltd.

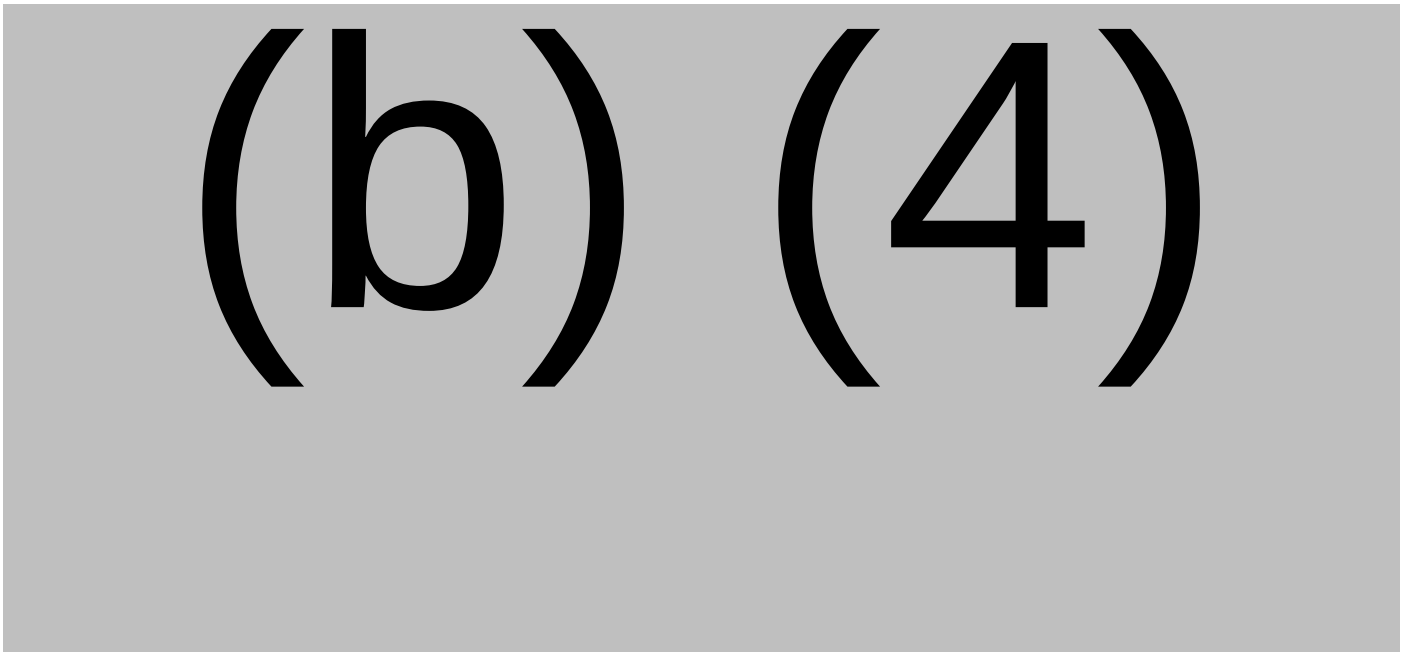
3. Food and Drug Administration (FDA). 2016. GRN 625. A GRAS notice for pyrroloquinoline quinone (PQQ) disodium salt, filed by Nascent Health.

4. Gao Y, 2016a. Mutagenicity, genotoxicity and sperm health studies of PQQ disodium salt -Appendix G

5. Nakano M, Suzuki H, Imamura T, Lau A, Lynch B. Genotoxicity of pyrroloquinoline quinine (PQQ) disodium salt (BioPQQ™). Regul Toxicol Pharmacol. 2013;67:189-97.

6. Urakami T, Yashima K, Kobayashi H, Yoshida A, Ito-Yoshida C. Production of pyrroloquinoline quinone by using methanol-utilizing bacteria. Appl Environ Microbiol. 1992;58:3970-6.

6 PAGES REDACTED UNDER (B)(4)
INTERNAL STUDIES AND ANALYSIS



Reference for the Section 3.2.3.

1. Food and Drug Administration (FDA). 2007. RPT 417. A NDI notice for pyrroloquinoline quinone (PQQ) disodium salt as dietary ingredient for dietary supplements, filed by Mitsubishi Gas Chemical Co., Inc.

2. Food and Drug Administration (FDA). 2016a. GRN 641. A GRAS notice for pyrroloquinoline quinone (PQQ) disodium salt, filed by Zeijang Hisun Pharmaceutical Co, Ltd.

3. Food and Drug Administration (FDA). 2016b. GRN 625. A GRAS notice for pyrroloquinoline quinone (PQQ) disodium salt, filed by Nascent Health.

4. Gao Y, 2016a. Mutagenicity, genotoxicity and sperm health studies of PQQ disodium salt -Appendix G

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3.2.4. Human clinical studies

3.2.4.1. Studies published since the FDA's 2007 review of RPT 417

Recently published human clinical studies show that oral consumption of PQQ disodium salt at daily doses up to 60 mg per person is not associated with any adverse effects (Table 11; Harris et al., 2013; Itoh al., 2016; Nakano et al., 2009, 2015a, 2015b; Rucker et al., 2009). Unpublished human studies reported in RPT 417 were published by Nakano et al. in 2015 (2015a, 2015b). Also, part of the studies described in Rucker et al. (2009) (20 mg/day for 4 weeks) were also reported in the previous NDI notice, RPT 417. Thus, these studies are included in both Table 12 (Human clinical studies published since FDA's 2007 review) and Table 12 (Human clinical studies included in the FDA's 2007 review). Findings from human clinical studies published since the FDA's last review of 2007 are not inconsistent with the FDA's prior decision (Tables 11 and 12).

Table 11. Human clinical studies of PQQ disodium salt published since the FDA's 2007 review

Subjects	Dose	Duration	Measured Outcome	Results	Author
41 elderly healthy subjects	20 mg	12 wk	Cognitive function	No abnormal blood or urinary adverse events, nor adverse internal or physical examination findings at any point in the study. PQQ improved cognitive functions.	Itoh et al., 2016
29 healthy subjects, 40-57 y	20 mg	6 and 12 wk	Serum lipid profile	PQQ marginally significantly decreased serum LDL-cholesterol concentration.	Nakano et al., 2015a
22 healthy women with mildly dry skin, 20-49 y	20 mg	8 wk	Skin health	PQQ improved skin conditions.	Nakano et al., 2015b
17 healthy adults	20 mg	8 wk	Subjective symptoms, body weight, heart rate, blood	No adverse effects related to PQQ disodium salt, as measured by subjective symptoms, objective findings and	Nakano et al., 2012

			pressure	abnormal changes in the measured values were observed.	
20 healthy adults	0, 20, or 60 mg/d	4 wk	Liver toxicity, urinary biomarkers, serum biochemistry, and adverse events	No adverse effects were observed in standard clinical tests (glucose, triglycerides, lipoprotein fractions). Functional tests for liver toxicity were also normal. Urinary N-acetyl-β-(D)-glucosaminidase activity was also normal. No adverse effects of PQQ disodium salt were noted.	Tsuji et al., 1998, Urakami et al., 1994; cited in Rucker et al., 2009
71 healthy adults, 45-65 y	20 mg PQQ+ 300 mg CoQ10	4, 8, and 12 wk	Memory and attention	PQQ significantly increased word memorization, recall task and attention (no data shown).	Nakano et al., 2009

Itoh et al. (2016) examined the effect of PQQ disodium salt on cognitive function in 41 elderly healthy subjects. Subjects were orally given 20 mg of PQQ disodium salt per day or a placebo for 12 weeks. For assessment of cognitive function, selective attention by the Stroop and reverse Stroop test, and visual-spatial cognitive function by the laptop tablet Touch M, were evaluated. The Stroop and reverse Stroop test examine selective attention ability, which are frequently used to examine individual differences in cognitive ability. Touch M is a simple evaluation system for visual-spatial cognitive function utilizing a laptop tablet. In the Stroop test, the change of Stroop interference ratios (SIs) for the PQQ group was significantly smaller than for the placebo group. It was expected that the SIs and RIs would decrease between 0 weeks and 12 weeks only for the PQQ group if the 12-week intake of the substance improves participants' cognitive ability. However, such a decrease in either the SIs or the RIs was not observed, indicating no effects of PQQ on such cognitive ability. In the Touch M test, the stratification analyses dividing each group into two groups showed that only in the group of the PQQ group with initial scores of less than 70 had significantly increased scores after 12 week intervention. Measurements of physiological parameters indicated no abnormal blood or urinary adverse events, nor adverse internal or physical examination findings at any point in the study. The preliminary experiment using near-infrared spectrometry (NIRS) suggests that cerebral blood flow in the prefrontal cortex was increased by the administration of PQQ. The results suggest that PQQ can prevent reduction of brain function in aged persons, especially in attention and working memory.

Nakano et al. (2015a) investigated the effects of PQQ disodium salt on serum triglyceride (TG) and cholesterol levels in humans after 6 and 12 weeks of treatment at an oral dosage of 20 mg/day. A total of 29 healthy adults, ranging from 40 to 57 years old, with normal to moderately

high TG levels (110-300 mg/dL) were included in this study. In eleven out of 29 volunteers, serum low-density lipoprotein cholesterol (LDL-C) levels at baseline was high (≥140 mg/dL). After 12 weeks, the mean serum concentrations of TG, total cholesterol (TC) and high density lipoprotein-cholesterol (HDL-C), body fat and body mass index (BMI) were not significantly changed. However, the mean LDL-C concentration was decreased with marginal significance, though not statistically significant (from 136.1 to 127.0 mg/dL, P=0.08) in the PQQ group. In the stratification analysis of the high LDL-C subgroup (baseline LDL-C level ≥140 mg/dL), the mean LDL-C levels decreased significantly from the baseline values in the PQQ group. Blood clinical biochemistry tests (aspartate transaminase [AST], alanine aminotransferase [ALT], glutamyl transpeptidase [GTP], lactate dehydrogenase [LDH], leucine transpeptidase, zinc sulfate turbidity test, total bilirubin, cholinesterase, urea nitrogen, total protein, creatine, calcium, serum iron, serum amylase, glucose) revealed that all the values were within normal ranges. In addition, no clinically significant changes were observed after the 12-week intervention, indicating the safety of PQQ. No adverse effects of PQQ were reported on the measured outcomes.

In a human study by Nakano et al. (2015b), oral intake of PQQ disodium salt (20 mg/d for 8 weeks) had a tendency to inhibit the increase in transepidermal water loss on the forearm (P>0.05) in 22 healthy female subjects (22-49 years old) with a subjective symptom of dryness in their arms. Subject questionnaires showed positive impressions for the improvement of skin conditions including dryness, hydration, softness, viscoelasticity and coarseness of the arm, face and body. These results suggest that oral intake of PQQ improved skin conditions in female subjects with dry skin. The authors noted no adverse effects of PQQ during the study.

In a double-blind, placebo-controlled clinical trial with 71 middle-aged and elderly people aged between 40-70 years, supplementation with 20 mg per day of PQQ resulted in improved cognitive function tests compared to the placebo group, but in the group receiving 20 mg of PQQ along with 300 mg of Coenzyme Q10 (CoQ10), the results were even more profound (data not shown; Nakano et al., 2009). The data demonstrated that, while PQQ is somewhat effective on its own, when it is combined with CoQ10, even better results have been shown. PQQ and CoQ10 are both involved in mitochondrial energy production.

Tsuji et al. (1998; cited in Rucker et al., 2009) and Urakami et al. (1994; cited in Rucker et al., 2009) performed double-blinded safety studies for PQQ in preparation for human use patents. PQQ was administered at 20 or 60 mg/day for four weeks to two groups of 10 healthy adults each (n=20), who were given either PQQ or a placebo. No adverse effects were observed in standard clinical tests (glucose, triglycerides, lipoprotein fractions). Functional tests for liver toxicity were also normal (aspartate aminotransferase and serum glutamic oxaloacetic transaminase). Urinary N-acetyl-β-(D)-glucosaminidase activity was also normal. No adverse effects of PQQ were noted.

It should be noted that part of the studies described in Rucker et al. (2009) (20 mg/day for 4 weeks) were reported in the previous NDI notice, RPT 417.

3.2.4.2. Studies included in the FDA's 2007 review

As previously discussed, unpublished human studies reported in RPT 417 were published by Nakano et al. in 2015 (2015a, 2015b; Table 13). It should be noted that part of the studies described in Rucker et al. (2009) (20 mg/day for 4 weeks) were also reported in the previous NDI notice, RPT 417 (FDA, 2007). PQQ disodium salt was administered at 20 mg/day for four weeks to two groups of 10 healthy adults each (n=20), who were given either PQQ disodium salt or a placebo (FDA, 2007; RPT 417). No adverse effects were observed in standard clinical tests (glucose, triglycerides, and lipoprotein fractions). Functional tests for liver toxicity (aspartate aminotransferase and serum glutamic oxaloacetic tranaminase), as well as urinary N-acetyl-β-(D)-glucosaminidase activity were also normal. No adverse effects of PQQ disodium salt were noted in these studies.

Table 12. Human clinical studies included in the 2007 FDA's review

Subjects	Dose	Duration	Measured Outcome	Results	Author
20 healthy males and females	20 mg PQQ (form, not specified)	4 wk	Liver toxicity, urinary biomarkers, serum biochemistry, and adverse effects	No adverse events were observed. No treatment-related abnormalities in clinical tests, liver functional tests and urinary biomarkers.	FDA, 2007; RPT 417
29 healthy subjects, 40-57 y	20 mg PQQ disodium salt	6 and 12 wk	Serum lipid profile	PQQ marginally significantly decreased serum LDL-cholesterol concentration.	FDA, 2007; RPT 417
22 healthy women with mildly dry skin, 20-49 y	20 mg PQQ (form, not specified)	8 wk	Skin health	PQQ improved skin conditions	FDA, 2007; RPT 417

Abbreviations: PQQ = pyrroloquinoline quinone; wk= weeks; y=years

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3.2.5. Discussion of toxicity and conclusion

Animal toxicity studies suggested that the NOAEL for PQQ disodium salt may be higher than 400 mg/kg bw/day in rats (Liang et al., 2015). After considering the safety margin of 100, it is reasonable to conclude that doses of up to 4 mg/kg bw/day could be expected to be safe in humans. This level corresponds to 332 mg/person/day for an average American adult (Please note that 83 kg was used as the average American adult body weight for this calculation). Intended use level (30 mg/person/day) is over 10 times lower than this estimated safe intake level.

Section 4. Basis for Concluding that the New Dietary Ingredient Will Reasonably Be Expected To Be Safe For Use as a Dietary Supplement

4.1 Determination of the No-Observed-Adverse-Effect-Level (NOAEL) or Lowest-Observed Adverse Effect Level (LOAEL)

Animal toxicity studies showed that the NOAEL for PQQ disodium salt is 400 mg/kg bw/day in rats (Liang et al., 2015). After considering the safety margin of 100, it is reasonable to conclude that 4 mg/kg bw/day may be safe in humans; this level corresponds to 332 mg/person/day in an American adult (given that the average American adult has a body weight of 83 kg). Intended use level (30 mg/person/day) is far below this level.

4.2 Determination of a Safety Factor

A safety margin of 100 has been applied to the NOAEL value found from a subchronic toxicity study in rats.

4.3 Determination of the Acceptable Daily Intake (ADI)

No acceptable daily intake (ADI) is available at this time. However, based upon the NOAEL value of 400 mg/kg bw/day, taking into account a safety factor of 100-fold, the daily dose of PQQ disodium salt up to 4.0 mg/kg bw/day as a dietary supplement is reasonably expected to be safe for human adults. This level corresponds to 332 mg per person for an average American adult (i.e. an adult with a body weight of 83 kg).

4.4 Determination of Estimated Daily Intake (EDI) and the EDI/ADI Ratio

Not applicable.

4.5 Determination of a Margin of Safety

A typical safety margin of 100 has been applied to the NOAEL value found from oral subchronic toxicity studies in rats.

4.6 Safety Narrative and Conclusion

PQQ is naturally occurring in various foods and beverages including human milk, and is detected in various organs and tissues of humans. Most of the PQQ administrated orally is excreted via urine within 24 h in the mouse model. A series of mutagenicity and genotoxicity studies report that PQQ is not mutagenic and/or genotoxic. Oral subchronic toxicity tests in rats found that NOAEL was higher than 400 mg/kg/day, the highest level tested. Based upon the evidence described above, taking into account a safety factor of 100-fold, the daily dose of PQQ disodium salt up to 4.0 mg/kg bw/day as a dietary supplement is reasonably expected to be safe

for human adults. This level corresponds to 332 mg per person for an average American adult. Nutraland's intended maximum use level of 30 mg/person/day is 10 times lower than the estimated safe intake level of 332 mg/person/day in adults.

Children and pregnant women are not recommended to consume PQQ disodium salt as a dietary supplement due to no available safety data at this time.

Nutraland's PQQ will replace currently marketed PQQ disodium salt or other PQQ products. Thus, cumulative exposures are not expected.

4.7 Alternative basis for Reasonable Expectation of Safety

PQQ disodium salt supplementation at a maximum daily dose of 60 mg per person has been safely consumed in the past 2 decades. The FDA has previously issued a 'no objection' letter on the NDI notification (RPT 417) related to PQQ filed by Mitsubishi Gas Chemical Co., Inc. The maximum intended use level proposed in RPT 417 was 60 mg/person/day, which is much higher than the level proposed in this notice (30 mg/person/day).

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