SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 24 October 2017

doi: 10.2903/j.efsa.2017.5058

Safety of pyrroloquinoline quinone disodium salt as a novel food pursuant to Regulation (EC) No 258/97

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), Dominique Turck, Jean-Louis Bresson, Barbara Burlingame, Tara Dean, Susan Fairweather-Tait, Marina Heinonen, Karen Ildico Hirsch-Ernst, Inge Mangelsdorf, Harry J McArdle, Androniki Naska, Monika Neuhäuser-Berthold, Grażyna Nowicka, Kristina Pentieva, Yolanda Sanz, Alfonso Siani, Anders Sjödin, Martin Stern, Daniel Tomé, Marco Vinceti, Peter Willatts, Karl-Heinz Engel, Rosangela Marchelli, Annette Pöting, Morten Poulsen, Josef Rudolf Schlatter, Agnès de Sesmaisons and Henk Van Loveren

Abstract

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on pyrroloquinoline quinone disodium salt (PQQ), trade name BioPQQ™, as a novel food pursuant to Regulation (EC) No 258/97. PQQ is produced by fermentation using *Hyphomicrobium denitrificans* CK-275 and purification process. PQQ has a minimum purity of 99.0%. The information provided on the composition, specifications, batch-to-batch variability, stability and production process of PQQ is sufficient and does not raise safety concerns. The applicant intends to market PQQ for use in food supplements for healthy adults, except pregnant and lactating women, at a maximum proposed level of consumption of 20 mg/day (corresponding to 0.29 mg/kg bw per day for a 70-kg person). The proposed level of consumption is at least 250 times higher than the estimated background intake of PQQ occurring naturally in foods. Information on the absorption, distribution, metabolism and excretion of PQQ in animals and humans is limited. Considering the no-observed-adverse-effect-level (NOAEL) of 100 mg/kg bw per day from a 90-day repeated dose oral toxicity study with BioPQQ™, and the maximum proposed level of consumption, the Panel concludes that the margin of exposure (of 344) is sufficient. The Panel concludes that the novel food, pyrroloquinoline quinone disodium salt (BioPQQ™), is safe under the intended conditions of use as specified by the applicant.

Keywords: pyrroloquinoline quinone disodium salt, novel food, ingredient, safety

Requestor: European Commission following an application by Mitsubishi Gas Chemical Company, Inc.

Question number: EFSA-Q-2016-00659

Correspondence: nda@efsa.europa.eu

Panel members: Jean-Louis Bresson, Barbara Burlingame, Tara Dean, Susan Fairweather-Tait, Marina Heinonen, Karen Ildico Hirsch-Ernst, Inge Mangelsdorf, Harry J McArdle, Androniki Naska, Monika Neuhäuser-Berthold, Grażyna Nowicka, Kristina Pentieva, Yolanda Sanz, Alfonso Siani, Anders Sjödin, Martin Stern, Daniel Tomé, Dominique Turck, Henk Van Loveren, Marco Vinceti and Peter Willatts.

Suggested citation: EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), Turck D, Bresson J-L, Burlingame B, Dean T, Fairweather-Tait S, Heinonen M, Hirsch-Ernst KI, Mangelsdorf I, McArdle HJ, Naska A, Neuhäuser-Berthold M, Nowicka G, Pentieva K, Sanz Y, Siani A, Sjödin A, Stern M, Tomé D, Vinceti M, Willatts P, Engel K-H, Marchelli R, Pöting A, Poulsen M, Schlatter JR, de Sesmaisons A and Van Loveren H, 2017. Scientific Opinion on the safety of pyrroloquinoline quinone disodium salt as a novel food pursuant to Regulation (EC) No 258/97. EFSA Journal 2017;15(11):5058, 19 pp. https://doi.org/10.2903/j.efsa.2017.5058

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Summary

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on the safety of pyrroloquinoline quinone disodium salt (PQQ), trade name BioPQQ™, as a novel food (NF) pursuant to Regulation (EC) No 258/97. The assessment, which follows the methodology set out in Commission Recommendation 97/618/EC, is based on the data supplied in the original application, the initial assessment by the competent authority of Ireland, the concerns and objections of a scientific nature raised by the other Member States and the responses of the applicant.

PQQ is produced by fermentation using *Hyphomicrobium denitrificans* CK-275 and purification process. The final form of the PQQ is a reddish-brown powder with a minimum purity of 99.0%. The source organism does not have a history of use in food. Notwithstanding the limited knowledge on the bacterial strain, neither the live bacteria nor the DNA are present in the NF above the detection limits of the analytical techniques applied. *H. denitrificans* is included in the microbiological specifications of the NF. The Panel therefore considers that the use of *H. denitrificans* does not raise concerns about the safety of PQQ.

The information provided on the composition, specifications, batch-to-batch variability, stability and production process of PQQ is sufficient and does not raise safety concerns.

The applicant intends to market PQQ for use in food supplements for healthy adults, except pregnant and lactating women, at a maximum proposed level of consumption of 20 mg/day (corresponding to 0.29 mg/kg bw per day for a 70-kg person). Food supplements containing PQQ are not intended for use by children. PQQ is naturally present at low levels in various food products. The proposed level of consumption is at least 250 times higher than the estimated background intake of PQQ occurring naturally in foods.

Taking into account the composition and the intended use levels of PQQ, the Panel considers that the consumption of PQQ is not nutritionally disadvantageous.

Information on the absorption, distribution, metabolism and excretion of PQQ in animals and humans is limited.

An *in vitro* bacterial reverse mutation assay, three *in vitro* chromosomal aberration tests and one *in vivo* micronucleus test were conducted with PQQ. Based on these studies, the Panel concludes that there is no concern with regard to potential genotoxicity of PQQ.

Twelve clinical studies were conducted on PQQ with doses up to 100 mg/day for up to 24 weeks. These studies do not raise safety concern. The Panel notes, however, that these studies were not designed to assess renal function and are of limited value for the safety assessment.

A 14-day dose-range finding study, a 90-day repeated-dose toxicity study and a 28-day renal toxicity have been conducted in rats using BioPQQ™. A 90-day repeated-dose toxicity study was also conducted using a PQQ product from another manufacturer with 98% purity. The Panel considers the findings of crystal and protein in urine at 200 mg/kg bw in the 28-day toxicity study as the critical effect based on the fact that renal toxicity was observed at a concentration of 768 mg/kg bw in the 14-day study. Therefore, the Panel considers 100 mg/kg bw from the 90-day study on BioPQQ™, which included a thorough urinalysis, as the overall no-observed-adverse-effect-level (NOAEL).

Considering the NOAEL of 100 mg/kg bw per day and the maximum proposed level of consumption, the Panel concludes that the margin of exposure (of 344) is sufficient.

The Panel concludes that the NF, pyrroloquinoline quinone disodium salt (BioPQQ™), is safe under the intended conditions of use as specified by the applicant.

Table of contents

1. Introduction

1.1. Background and Terms of Reference as provided by the European Commission

On 6 December 2012, the company Mitsubishi Gas Chemical Company, Inc. submitted a request in accordance with Article 4 of the Novel Food Regulation (EC) No 258/97 to place on the market pyrroloquinoline quinone disodium salt (PQQ) as a novel food ingredient.

On 8 July 2016, the competent authority of Ireland forwarded to the Commission its initial assessment report, which came to the conclusion that an additional assessment is required in accordance with Article 6.3 of the novel food Regulation (EC) No 258/97 for pyrroloquinoline quinone disodium salt.

On 2 August 2016, the Commission forwarded the initial assessment report to the other Member States. The Member States agreed with the initial assessment report made by the Food Safety Authority of Ireland and some of them included additional comments.

In consequence, a decision is now required by the Commission under Article 7(1) of Regulation (EC) No 258/97.

The concerns of a scientific nature raised by the Member States can be summarised as follows:

- Concerning the data provided in relation to the product specifications, it was requested whether the tests were performed by laboratories that are accredited for the methods used.
- Quantitative data on the content of PQQ in foods were required to estimate natural exposure in comparison with the intended use.
- With respect to the monitoring of microbiological contamination, information concerning the sampling plans was considered insufficient. In the case of plans involving two categories that require the absence of germs, the quantity of the product examined and the scope of the sampling should be stated.
- It was highlighted that the antioxidants pro-oxidant functions are greatly dose-dependent and the antioxidant–pro-oxidant system is in very sensitive balance.
- It was considered that the limited data on the physiological and metabolic functions associated with PQQ make it difficult to carry out a meaningful evaluation of any risks associated with long-term consumption of PQQ at the proposed dose levels.
- The complete reports of the toxicological studies should be provided in order to assess the published studies, in particular on genotoxicity.
- Considering the low background intake of PQQ in comparison with the proposed intake of the highly purified compound via food supplements and the possible long-term consumption of such food supplements by some consumers, a full toxicological assessment, including studies aimed at examining potential chronic toxicity and carcinogenicity, was considered necessary. Such research would also address remaining concerns regarding the reported observations in the genotoxicity studies.
- The results from the animal toxicological studies in relation to crystals of the novel ingredient occurring in the urine should be further investigated. The potential for this to be a concern unrelated to renal function was raised.
- The relevance for human exposure of the effects on the kidney should be evaluated further.
- The human studies provided cannot be used as a basis for an assessment because of their limited sample sizes and durations (maximum 24 weeks).

In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority to provide a scientific opinion by carrying out the additional assessment for Pyrroloquinoline Quinone Disodium Salt as a novel food ingredient in the context of Regulation (EC) No 258/97.

2. Data and methodologies

2.1. Data

The assessment of the safety of this novel food (NF) is based on data supplied in the original application, the initial assessment by the competent authority of Ireland, the concerns and objections of the other Member States and the responses of the applicant (see 'Steps taken by EFSA').

In accordance with Commission Recommendation 97/618/EC[1], pyrroloquinoline quinone disodium salt is allocated to Class 1.2, i.e. 'foods/food ingredients consisting of or isolated from microorganisms, fungi or algae. The source of the novel food has no history of food use in the Community'. The data are required to comply with the information required for novel foods of Class 1.2, i.e. structured schemes I, II, III, IX, XI, XII and XIII of Commission Recommendation 97/618/EC. In the current opinion, these structured schemes are listed in Sections 3.1–3.8. The intention is to add the NF to food supplements. This assessment concerns only the risk that might be associated with consumption of the NF under the proposed conditions of use, and is not an assessment of the efficacy of the NF with regard to any claimed benefit.

2.2. Methodologies

The assessment follows the methodology set out in Commission Recommendation 97/618/EC of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients and the preparation of initial assessment reports under Regulation (EC) No 258/97 of the European Parliament and of the Council.

3. Assessment

3.1. Specification of the novel food

Pyrroloquinoline quinone disodium salt (PQQ) is an ingredient manufactured by the company Mitsubishi Gas Chemical Company Inc., marketed under the trade name BioPQQ™. PQQ is an aromatic heterocyclic orthoquinone (Figure 1). Its chemical name is disodium 9-carboxy-4,5-dioxo-1*H*-pyrrolo [5,4-f]quinoline-2,7-dicarboxylate and its molecular formula is $C_{14}H_4N_2Na_2O_8$. It has a molecular weight of 374.17 Da. Its Chemical Abstracts Service (CAS) number is 122628-50-6.

PQQ was originally identified as a bacterial cofactor for primary alcohol dehydrogenases (Hauge, 1964; Anthony and Zatman, 1967; Salisbury et al., 1979; Duine and Jongejan, 1989). It has been reported to be produced by Gram-negative, methanol-utilising bacteria, such as strains of the genera *Hyphomicrobium* and *Methylobacterium* where PQQ is biosynthesised from L-tyrosine and L-glutamic acid precursors (Houck et al., 1988; van Kleef and Duine, 1988; Urakami et al., 1992).

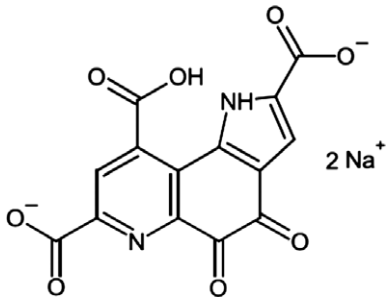

Figure 1: Chemical structure of pyrroloquinoline quinone disodium salt

The NF is produced by the bacterium strain *Hyphomicrobium denitrificans* CK-275 (Section 3.2). The final form of the NF is a reddish-brown powder with a minimum purity of 99.0%.

The specifications of the NF are provided in table 1. The specifications include a maximum limit for ethanol (< 500 ppm), which is used as a solvent during the manufacture of the NF. Upon request from EFSA, a limit for *H. denitrificans* has been included in the specifications.

Compositional data for five non-consecutive batches of the NF are provided in Table 2 and comply with the specifications. The applicant analysed the presence of *H. denitrificans* in three non-consecutive batches of the NF (H-0323A01, H-0423A01 and H-0823A01). Samples of the NF (1 g) were plated on agar plates of the medium required for the growth of the microorganism and incubated for 7 days at 37°C. No bacterial colonies were detected in any batch (detection limit: 25 CFU/g NF).

[1] Commission Recommendation 97/618/EC: Commission Recommendation of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients and the preparation of initial assessment reports under Regulation (EC) No 258/97 of the European Parliament and of the Council. OJ L 253, 16.9.1997, p. 1–36.

The applicant confirmed that the facility, Mitsubishi Gas Chemical Company Niigata factory, involved in the batch analysis testing (Mitsubishi Gas Chemical Co., Inc.) is certified in accordance with ISO 9001:2008 and ISO 14001:2004.

Table 1: Specifications for PQQ

Parameter	Specification	Method
Identity		
Appearance	Reddish-brown powder	Visual inspection
Purity		
Purity	> 99.0%	HPLC
UV absorbance (A322/A259)	0.56 ± 0.03	Spectrophotometry (JFSL method B37)
UV absorbance (A233/A259)	0.90 ± 0.09	Spectrophotometry (JFSL method B37)
Moisture content	< 12%	Karl Fischer titration (JFSL method B43)
Residual solvent		
Ethanol	< 500 ppm	Gas chromatography (JFSL method B14)
Heavy metals		
Lead	< 3 ppm	Atomic absorption (JFSL method B03)
Arsenic	< 2 ppm	Atomic absorption (JFSL method B03)
Microbiological specifications		
Total viable cell count	< 300 CFU/g	JFSL method B26
Coliforms	Absent in 1 g	JFSL method B26
Fungi	< 12 CFU/g	JFSL method B26
Hyphomicrobium denitrificans	< 25 CFU/g	Plate counting

CFU: colony forming units; HPLC: high-performance liquid chromatography; JFSL: Japan Food Sanitation Law; UV: ultraviolet.

Table 2: Batch-to-batch analyses of PQQ

Parameter	Specification	Batch results				
		H-1022A02	H-1122A01	H-1222A01	H-0223A01	H-0323A01
Identity						
Appearance	Reddish-brown powder	Reddish-brown powder	Reddish-brown powder	Reddish-brown powder	Reddish-brown powder	Reddish-brown powder
Purity						
Purity	> 99.0%	100.0%	99.96%	99.99%	100.0%	100.0%
UV absorbance (A322/A259)	0.56 ± 0.03	0.553	0.560	0.556	0.559	0.559
UV absorbance (A233/A259)	0.90 ± 0.09	0.868	0.870	0.869	0.866	0.869
Moisture content	< 12%	10.2%	8.78%	10.01%	10.80%	10.93%
Residual solvent						
Ethanol	< 500 ppm	ND	ND	ND	ND	ND
Heavy metals						
Lead	< 3 ppm	0.052 ppm	ND	ND	ND	ND
Arsenic	< 2 ppm	< 2 ppm	< 2 ppm	< 2 ppm	< 2 ppm	< 2 ppm
Microbiological specifications						
Total viable cell count	< 300 CFU/g	ND	ND	ND	ND	ND
Coliforms	Absent in 1 g	ND	ND	ND	ND	ND
Fungi	< 12 CFU/g	ND	ND	ND	ND	ND

CFU: colony forming units; ND: not detected; UV: ultraviolet.

In response to EFSA's request regarding the purpose of the use of UV absorbance measures at two wavelengths to determine the purity of the NF, the applicant indicated that the ratio between the absorbance at the two wavelengths (A233/A259 and A322/A259) allows the differentiation between the oxidised and reduced forms of PQQ (i.e. in the reduced form the ratios of A233/A259 and A322/A259 are 1.11 and 1.62, respectively, whereas these values are different for the oxidised from of PQQ) as described elsewhere (Dekker et al., 1982). The two values are confirmatory of the identity and purity.

A crude sample of unknown purity was analysed by high-performance liquid chromatography/mass spectrometry (HPLC/MS) to identify potential impurities. The majority of the compounds identified are assumed to be quinone related and none of them was present at a level greater than 0.05%.

The applicant investigated whether cobalt may have been carried over in the final product from the use of cobalt chloride in the fermentation medium. Certificates of analysis for residual cobalt for six non-sequential batches of the final product were provided. The analyses were conducted using an atomic absorption method with a limit of quantitation of 0.05 ppm. The reported levels for cobalt ranged from not detected (five batches) to 0.07 ppm (one batch).

Upon request from EFSA, the applicant provided data on the NF solubility. Between 1.8 and 10.2 g of NF were dissolved in water at pH 2.95–4.92 (tested at temperature between 5°C and 80°C) and ca. 15 g of NF were dissolved in water at pH 5.66–9.75 (tested at temperature 25°C).

The Panel considers that the information provided on the composition, the specifications and the batch-to-batch variability of the NF is sufficient and does not raise safety concerns.

3.1.1. Stability of the NF

The stability of the NF in powder form (one lot) was assessed for up to 36 months in the following storage conditions: in the dark at −20, 5, 30 or 50°C; under light at 30°C; in a tightly closed or open container at 40°C and 75% relative humidity (accelerated conditions). The PQQ content of the powder, assessed via HPLC analysis, was stable (PQQ > 99%) under all of the storage conditions for up to 36 months.

The stability of the NF in aqueous solutions was also assessed in water (pH 3.6) and in a 50 mM potassium phosphate buffer (pH 6). The solutions, each containing 1 g PQQ/L, were stored in the dark at −20, 5, 30 or 50°C, or under light conditions at 30°C, for up to 36 months. When stored in the dark at temperatures ≤ 30°C, the NF was stable in aqueous solutions at pH 3.6 and 6 for at least 36 months (ca. 99% PQQ, assayed by HPLC). In contrast, when stored at 50°C in the dark and at 30°C in the light, the PQQ content gradually decreased down to 1.38% (dark, 50°C) and 3.51% (light, 30°C) at pH 3.6 and 49.82% (dark, 50°C) and 45.81% (light, 30°C) at pH 6.0, after 3 years of storage.

In response to EFSA's request regarding the rationale for testing the stability of the NF in solutions and under different pH conditions, the applicant indicated that the stability of the NF was tested in these conditions to evaluate the effect of physical form, although it intends to use PQQ only in the powder form.

The Panel considers that the data provided sufficient information with respect to the stability of the NF.

3.2. Effect of the production process applied to the NF

The NF is produced according to current Good Manufacturing Practices (cGMP) principles during a Hazard Analysis Critical Control Points (HACCP)-controlled fermentation using the non-genetically modified microorganism *Hyphomicrobium denitrificans* CK-275. During the fermentation process, PQQ is secreted by the bacteria into the fermentation broth. Particulate material is removed by centrifugation from the fermentation broth. PQQ is then purified through a series of acid and salt precipitation steps, pH adjustment, filtration and anion-exchange column chromatography. The resultant slurry with ethanol is filtered and the cake vacuum dried (at 50°C for several hours), sieved to a powder form. The manufacturing process results in a highly purified product (> 99%). The NF is then stored in aluminium lined bags.

All raw materials and processing aids used in the manufacture of the NF are of food-grade quality. The applicant indicates that the processing aids used in the manufacture are removed during the purification steps. Ethanol, which is used as a solvent in the production process, was shown to be absent from the final product (Section 3.1).

The Panel considers that the production process is sufficiently described and does not raise safety concerns.

3.3. History of the organism used as the source of the NF

H. denitrificans does not have a history of use in food. The production strain CK-275 is obtained from the National Institute of Technology and Evaluation, Biological Resource Center, Japan.

H. denitrificans is an aerobic, facultatively methylotrophic, Gram-negative, rod-shaped non-spore-forming organism, whose major ubiquinone is coenzyme Q9 (Urakami et al., 1995; HAMAP, 2011). The strain has been deposited in the American type culture collection (ATCC 51888), the German collection of microorganisms and cell cultures (DSM 1869) and the UK national collection of industrial bacteria (NCIB 11706). The strain (ATCC 51888) has been characterised by whole-genome sequencing (Brown et al., 2011). It has been classified as 'risk group 1' in the classification of the German collection of microorganisms and cell cultures (DSMZ), which covers microorganisms that are unlikely to cause disease in humans (ABAS, 2015).

No safety concerns or reports on antimicrobial resistance have been described for the species *H. denitrificans* according to a recent assessment done by the EFSA BIOHAZ Panel. However, it has been concluded that the body of knowledge is limited and, therefore, this bacterial species cannot be taken into consideration for being included in the Qualified Presumption of Safety (QPS) list (EFSA BIOHAZ Panel et al., 2017).

The applicant conducted two acute toxicity studies to evaluate the safety of *H. denitrificans* in mice and rats (Kikuchi, 2011, 2012).

In one study, male ICR mice (16/group) received an intravenous administration of the production strain (CK-275, live bacteria) at doses ranging from 7.4×10^6 to 7.4×10^9 cells (as determined by CFU on plates) per animal (Kikuchi, 2011). Animals were monitored for mortality, body weight change and general signs and symptoms of toxicity for 33 days following injection. No death occurred during the course of the study. The acute intravenous median lethal dose (LD_{50}) was estimated to be greater than 7.4×10^9 (viable bacterial cell count) per animal. Upon necropsy, splenomegaly was observed on day 6 in the groups with 7.4×10^7 and 10^8 cells per animal, and on days 6 and 13 in the group with 7.4×10^9 cells per animal. Severity of splenomegaly was considered mild in most of the animals, while in two animals of the highest dose group, spleen size was 1.5–3 times larger than the normal size. It was considered to be a physiological reaction which occurs commonly with increased capturing process of foreign substances. No abnormality was detected on days 21 and 33 in any group. According to intra-organ viable cell count of brain, lungs, liver, kidneys and spleen, using the Stamp method, there was no survival or proliferation of bacterial cells. The Panel notes that limited conclusions can be drawn from a study using intravenous administration on the effect of an oral intake of the producer organism.

In an acute oral toxicity study reported to be conducted in accordance with the Organisation for Economic Cooperation and Development (OECD) Guidelines for the Testing of Chemicals No 423 (OECD, 2011), female Sprague–Dawley rats received a 50% (w/v) suspension of the production strain (CK-275, live bacteria) via gavage (Kikuchi, 2012). The acute oral LD_{50} of the production strain was reported to be greater than 10 g/kg bw (equivalent to 7.5×10^9 CFU/kg bw). The Panel notes that limited conclusions can be drawn from an acute study with a unique dose on the effect of the sustained consumption of the producer organism.

The Panel notes that *H. denitrificans* does not have a history of use in food and that the body of knowledge on the bacterial strain is limited. Notwithstanding the limited knowledge on the bacterial strain, the Panel notes that neither the live bacteria nor the DNA are present in the NF above the detection limits of the analytical techniques applied (Sections 3.1 and 3.7). *H. denitrificans* is included in the microbiological specifications of the NF (< 25 CFU/g) (Section 3.1). The Panel therefore considers that the use of *H. denitrificans* as the producer organism does not raise concerns about the safety of the NF.

3.4. Anticipated intake/extent of use of the NF

3.4.1. Proposed conditions of use

The applicant intends to market the NF for use in food supplements for healthy adults. The maximum proposed level of consumption is 20 mg/day. Food supplements containing PQQ are not intended for use by children, pregnant and lactating women. The applicant indicates that the food supplement will be labelled according to Article 6(3) of Directive 2002/46/EC.[2]

[2] Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements. OJ L 183, 12.7.2002, p. 51–57.

3.4.2. PQQ intake from background diet

PQQ occurs naturally in various food products, including foods from plant origin (e.g. potato, spinach, parsley, green pepper), fermented food products (e.g. fermented soybeans (natto), tofu, miso) and foods originating from animals (e.g. milk, egg). Typical free PQQ contents in selected food products were found to range between 1 and 30 ng/g and reached up to 61 ng/g in natto (Kumazawa et al., 1993, 1995; Stites et al., 2000; Noji et al., 2007). Besides, PQQ derivatives such as imidazolopyrroloquinoline quinone (IPQ) are also present in foods, as a result of its reaction with amino acids and other nucleophiles (Mitchell et al., 1999; Noji et al., 2007). The applicant refers to papers which provide estimated intake of PQQ and its derivatives from foods between 0.1 to 1 mg/day (Harris et al., 2013) and 1 and 2 mg/day (0.1–0.3 mg/day free PQQ) (Rucker et al., 2009). The Panel notes that these publications do not describe the method used to derive these estimates.

Considering the typical contents of free PQQ in foods reported in the literature, the Panel notes that the intake of free PQQ from the background diet is at least 250 times lower than the proposed intake from the NF (based on a conservative estimate considering 2.6 kg[3] of daily food consumption containing 30 ng free PQQ/g food: 0.08 mg/day).

Trace amounts of free PQQ were detected in rat and human tissues at concentrations up to 5.9 ng/g wet tissue (analysed by gas chromatography/mass spectrometry). In human tissues, the highest levels were found in the spleen, pancreas, lung and kidney, while no PQQ was detected in the brain or heart (Kumazawa et al., 1992). Mitchell et al. (1999) reported combined concentrations of PQQ and IPQ in human milk samples between 140 and 180 ng/mL. In the absence of a known mammalian biosynthetic pathway for PQQ, the endogenous tissue levels of PQQ in humans are likely derived from dietary exposure (Kumazawa et al., 1995).

3.4.3. PQQ intake from food supplements and fortified foods

Dietary supplements containing PQQ are available in the USA and Canada for use by healthy adults at a maximum intake level of 50 mg/day and 20 mg/day (Health Canada, 2016), respectively.

3.5. Nutritional information on the NF

Taking into account the composition and the intended use levels of the NF, the Panel considers that the consumption of the NF is not nutritionally disadvantageous.

3.6. Microbiological information on the NF

No bacteria were detected in three non-consecutive batches of the NF (Section 3.1). Amplicons of the *nirK* gene (1,084 bp or 290 bp) of *H. denitrificans* were not detected by conventional and nested polymerase chain reaction (PCR) analysis of samples (0.1 g) of the NF using specific primers with a detection limit of $\leq 10^2$ cell/g sample (5 pg DNA/g PQQ) established by nested PCR. This indicates that *H. denitrificans* is below this limit of detection in the final product.

Microbiological specifications of the NF are indicated in Table 1. Certificates of analyses were provided for five batches of the NF, and microbiological contents conformed with the specifications (Section 3.1).

The Panel considers that the microbiological information provided does not raise safety concerns.

3.7. Toxicological information on the NF

3.7.1. Absorption, distribution, metabolism and excretion (ADME)

In a study by Smidt et al. (1991), male Swiss-Webster mice (n = 5 per time point) were orally administered a single dose of 1.5 mg/kg bw of radiolabelled (^{14}C) PQQ. Based on the amount retained in the tissues, and amount in the urine and carbon dioxide, 3.3% of the administered dose was absorbed after 6 h and at least 62% (range 19–89%) was absorbed within 24 h. Approximately 81% (range 62–96%) of absorbed radioactivity was excreted in the urine within this timeframe, 10.7% was detected after 24 h in the kidneys, 3.7% in the carcass, 1.5% in the liver, 1.3% in the skin and 1.2% in the blood. No radioactivity was detected in the expired air.

[3] For a 70-kg adult. Total mean food (solid + liquid) consumption for adults in the surveys of the EFSA comprehensive food consumption database is 37.5 g/kg bw per day (EFSA Scientific Committee, 2012. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 2012;10(3):2579, 32 pp. https://doi.org/10.2903/j.efsa.2012.2579)

In a study aiming at investigating the effect of PQQ intake on markers of inflammation, Harris et al. (2013) assessed the urinary excretion and serum concentration of PQQ in 10 healthy adults (five men and five women) who received PQQ in a fruit-flavoured drink at increasing dose levels of 0.075, 0.15 and 0.3 mg/kg bw per day (corresponding to approximately 5.25, 10.5 and 21 mg PQQ/day for a 70-kg individual) over three consecutive 7-day periods. Serum PQQ concentration and urinary excretion of PQQ were measured at the end of each period. At each dose of PQQ, approximately 0.1% of the administered PQQ was recovered in the urine as free PQQ. Serum concentrations of free PQQ increased in response to PQQ dietary intake up to 14 nM at a dose of 0.3 mg/kg bw per day. In another experiment, five male and five female subjects (mean age of 28.1 years) received a single dose of 0.2 mg PQQ/kg bw in an orange drink (corresponding to 14 mg PQQ for a 70-kg individual). Blood and urine samples were collected at 0, 2, 4, 8, 24 or 48 h post-administration. Serum concentration of PQQ peaked at 2 h post-administration at 9 nM (~ 3.4 ng/mL). The rise and clearance of PQQ concentration in blood paralleled the changes in urine concentration. No analyses for PQQ derivatives were conducted in these studies.

No studies on the metabolic fate of PQQ were identified. In samples of biological fluids and amino acid-rich solutions, PQQ was reported to react with amino acids to form imidazole derivatives such as IPQ (Mitchell et al., 1999).

The Panel notes that information on the ADME of PQQ is limited in animals and humans.

3.7.2. Genotoxicity

A bacterial reverse mutation assay and three chromosomal aberration tests were conducted in accordance with the Japanese Guidelines for Genotoxicity Studies of Drugs (Notification No. 1604, November 1, 1999).

A bacterial reverse mutation assay (Ames test) was conducted in *Salmonella* Typhimurium strains TA98, TA100, TA1535, TA1537 and *Escherichia coli* strain WP2uvrA (Mitsubishi Gas Chemical Company Inc, 2005b; Nakano et al., 2013). Strains were treated with PQQ (99.6% purity; dissolved in dimethyl sulfoxide) at concentrations between 0 (solvent control) and 5,000 μg/plate in the absence of metabolic activation, and between 0 (solvent control) and 5,000 μg/plate in the presence of metabolic activation (S9 mix). No evidence of test article precipitation was observed. Depending on the strain and the presence or absence of metabolic activation, growth inhibition was observed above specific doses. No increase in the number of revertant colonies was observed at any doses, neither in the presence or absence of metabolic activation, in any of the strains examined.

In an *in vitro* chromosomal aberration test with lung fibroblasts from Chinese hamsters (CHL/IU) cells were treated with PQQ (99.7% purity; dissolved in distilled water) at concentrations of 0 (water negative control), 12.5, 25, 50, 100, 200 or 400 μg/mL for 6 and 24 h in the absence of metabolic activation (Mitsubishi Gas Chemical Company Inc, 2006d; Nakano et al., 2013). Cells were also treated with 0 (water negative control), 117.2, 234.4, 468.8, 937.5, 1,875 or 3,750 μg/mL for 6 h in the presence of metabolic activation (S9 mix). In the absence of S9 mix, very limited metaphase cells were available for scoring at the highest concentration tested (400 μg/mL) and this treatment group was excluded from evaluation. A statistically significant increase in the incidence of structural chromosomal aberrations (excluding gaps) was observed following treatment with 200 μg/mL for 6 h (4.0% vs 0.0% in the negative control). An increase in the incidence of structural chromosomal aberrations (excluding gaps) was also observed following treatment with 200 μg/mL for 24 h, which was not statistically significant (4.0% vs 1.0% in the negative control). Growth rates were 18% and 21% at the respective time points. In the presence of S9 mix, precipitation was noted at all concentrations tested and a statistically significant increase in the incidence of structural chromosomal aberrations (excluding gaps) was observed at 1,875 μg/mL (3.0%) and 3,750 μg/mL (5.5% vs 0.0% in the negative control). Growth rates were 76% and 74% at the respective doses. The Panel considers that the study provides some evidence of clastogenicity under the conditions of the assay.

The *in vitro* chromosomal aberration test was repeated using PQQ (99.3% purity) (Mitsubishi Gas Chemical Company Inc, 2008a; Nakano et al., 2013). Limited number of metaphase cells were available for scoring at the highest concentration of 400 μg/mL following 6 h of incubation, and at concentrations of 200 and 400 μg/mL following 24 h of incubation in the absence of metabolic activation. These plates were excluded from the evaluation. In the absence of metabolic activation, no significant differences in the incidence of chromosomal aberrations were observed at any doses tested compared to the negative control. In the presence of metabolic activation, the incidence of

chromosomal aberrations (excluding gaps) was 7.0% at the highest dose level tested (vs 0.5% in the negative control; not statistically significant) and the growth rate was 66%. This was deemed to be an equivocal result by the investigators due to precipitation of the test article.

An *in vitro* chromosomal aberration test was conducted in human peripheral blood lymphocytes (Mitsubishi Gas Chemical Company Inc, 2008b; Nakano et al., 2013). Human peripheral blood lymphocytes were treated with PQQ (99.3% purity; dissolved in water) at concentrations of 0 (water negative control), 234.4, 468.8, 937.5, 1,875 or 3,750 μg/mL in the absence and presence of metabolic activation for 3 h, and at concentrations of 0 (water negative control), 117.2, 234.4, 468.8, 937.5, 1,875 or 3,750 μg/mL in the absence of metabolic activation for 24 h. In cells treated for 24 h in the absence of metabolic activation, a limited number of metaphase cells were available for scoring at doses of 1,875 and 3,750 μg/mL, and thus, these test doses were excluded from evaluation. At concentrations of 234.4 and 3,750 μg/mL with metabolic activation (3 h) and 937.5 μg/mL without metabolic activation (24 h), only 150 cells were available for scoring. For all treatments, precipitation of the test material was seen at the two highest concentrations tested. No significant increase in the incidences of structural aberrations was observed in cells treated with PQQ compared to the negative control under any test conditions.

An *in vivo* micronucleus test was conducted in Crlj:CD1 (ICR) male mice (n = 6 per group). Mice were administered PQQ (99.7% purity; dissolved in 0.5% methylcellulose solution) by gastric intubation at a dose of 0 (vehicle control), 250, 500, 1,000 or 2,000 mg/kg bw over two doses separated by a 24-h interval (Mitsubishi Gas Chemical Company Inc, 2006c; Nakano et al., 2013). Mitomycin C was used as a positive control. No significant difference in the percentage of micronucleated polychromatic erythrocytes was observed in mice treated with PQQ compared to the negative control, whereas the positive control mitomycin C induced the expected statistically significant increase. There was no change in the percentage of polychromatic erythrocytes after treatment with PQQ up to the highest dose level.

Upon request from EFSA, the applicant indicated that no toxicokinetic investigations were performed in the study that could detect the presence of PQQ or its metabolites and determine whether the test substance or its metabolites reached the bone marrow. To demonstrate that the PQQ is absorbed and present systematically, the applicant referred to an ADME study in Swiss-Webster mice given radiolabelled (^{14}C) PQQ, which showed that approximately 81% of the absorbed radioactivity was excreted in the urine within 24 h (Smidt et al., 1991) (Section 3.7.1). In this study, 10.6% of the absorbed dose was detected in red blood cells after 6 h. The Panel considers that it is likely that PQQ or its metabolites reached the bone marrow.

The applicant also refers to a paper by Wang et al. (2012) which report a micronucleus test conducted in ICR mice, the text of which was only available in Chinese.

The Panel notes that the bacterial reverse mutation test was negative and that the clastogenicity observed in *in vitro* chromosomal aberration tests was not observed in the *in vivo* micronucleus test in mice. Even though the genotoxicity testing strategy is not fully in line with current EFSA recommendations (EFSA Scientific Committee, 2011), as no micronucleus assay *in vitro* was provided for testing clastogenic and aneugenic properties of the NF, the Panel considers that there is no concern with respect to genotoxicity of the NF.

3.7.3. Acute toxicity studies

In an acute toxicity study, Crl:CD(SD) rats (n = 10/sex per group) were orally administered by gavage 0 (control), 500, 1,000 or 2,000 mg/kg bw of the NF (BioPQQ™; purity > 99.1%) (Nakano et al., 2014). Animals were observed for mortality and clinical signs for 14 days after administration. High mortality was seen in the high-dose group where 6/10 males and 10/10 females died within the first three days. One female from the mid-dose group died during the 14-day observation period.

3.7.4. Subacute and subchronic toxicity studies

In a 14-day dose-range finding study, Crl:CD(SD) rats (n = 6/sex per group) were administered the NF (BioPQQ™; purity > 99.1%) by gavage at doses of 0 (control), 3, 12, 48, 192 or 768 mg/kg bw per day (Mitsubishi Gas Chemical Company Inc, 2005a; Nakano et al., 2014). No mortality was reported for any of the groups. Dark-green faeces were observed in males at 192 mg/kg bw per day, and in both sexes at 768 mg/kg bw per day, which was suggested to be due to the excretion of the test substance. There were no statistically significant differences in body weight among groups. Upon haematological analysis, a significantly lower mean cell volume was observed for males at 768 mg/kg bw.

In the absence of findings on other blood parameters, the Panel considers this as an incidental finding. Crystals were seen in the urine of some female rats at doses of 3, 12, 192 or 768 mg/kg bw per day (n = 1, 3, 2 and 1, respectively) and in one male at dose of 192 mg/kg bw per day. Protein levels in urine were increased (> 30 mg/dL) in one female at a dose of 12 mg/kg bw and one female at a dose of 192 mg/kg bw and two females and two males at a dose of 768 mg/kg bw per day. No crystals or increased protein levels were seen in the control groups of either sex. An increase in urinary sodium was observed in both sexes at the highest dose. A significant increase in relative kidney weights, which was accompanied by histopathological changes (focal basophilic changes and atrophy of the renal tubules) in the kidneys, was observed in high-dose females. The Panel considers that this indicates a toxic effect of PQQ on the kidneys at the high dose.

In a 90-day repeated-dose toxicity study, Crl:CD(SD) rats were administered 0 (control), 3, 20 or 100 mg/kg bw per day of the NF (BioPQQ™; purity > 99.1%) by gavage (n = 10/sex per group) (Mitsubishi Gas Chemical Company Inc, 2005a; Nakano et al., 2014). The study was conducted according to Japanese Good Laboratory Practice (GLP) for non-clinical laboratory studies on the safety of drugs and OECD Guidelines for the Testing of Chemicals No 408 (OECD, 1998), although no functional observation battery tests have been performed. No mortality or clinical signs of toxicity were observed in any group. Dark-green coloured faeces beginning on days 7–11 and thereafter were observed in high-dose males and females. This observation was attributed to the excretion of the unabsorbed test article. There were no compound-related adverse effects on body weight, food consumption, ophthalmology, organ weights (brain, pituitary gland, thyroid glands, heart, lungs with bronchi, salivary glands, liver, kidneys, thymus, spleen, adrenals, testes, seminal vesicle, prostate, ovaries, uterus) and haematology reported in any group.

A decrease (33%) in triglyceride concentrations in high-dose females was observed. In the absence of other findings on related parameters (e.g. body weight, growth and clinical chemistry), this is not regarded as an adverse effect.

Greenish-colouration of intestinal and caecal contents was observed in three high-dose males and one high-dose female; no gross or histopathological changes in the intestine, cecum, colon or rectum were detected. The histopathological findings in the liver, pancreas, salivary glands, stomach, kidneys, heart, lung and bronchi, thyroid glands, testes prostate, eyeball and optic nerve, mammary glands and Harderian glands were considered as normal physiological variations or sporadic changes since the frequencies were low and/or equal to those in the control group.

Urinalysis revealed increased protein levels > 100 mg/dL in one high-dose male and increased protein level between 30 and 100 mg/dL in one high-dose, two medium-dose, three low-dose and two control males. In females, increased protein level between 30 and 100 mg/dL was seen in two high-dose females. Crystals in urinary sediment were found in 1/10 males of the control group, 1/10 males in the low-dose group, 3/10 males in the medium-dose group and 2/10 males in the high-dose group and in 2/10 females in the high-dose group. No crystals were seen in the other female dose groups and controls. The Panel notes that these findings were not accompanied by significant changes in clinical chemistry or gross and histopathological investigations. Due to the comparable levels observed in the control group, the Panel considers that this is not an adverse effect.

The Panel concludes that the no-observed-adverse-effect-level (NOAEL) of the study is 100 mg/kg bw the highest dose tested.

In a more recent study, the subchronic oral toxicity of PQQ (Shanghai Med Co., Ltd., purity > 98%) was evaluated in rats (Liang et al., 2015). The full report of the study is not available to the Panel. This study was conducted in accordance with the US Food and Drug Administration (FDA) Principles of GLP and FDA Guidance on chronic toxicity studies with rodents for Industry and Other Stakeholders (US FDA, 2007). Groups of weanling Sprague–Dawley rats (10/sex per group; 60–80 g bw) were administered PQQ by oral gavage at doses of 0 (control), 100 (low dose), 200 (mid dose) or 400 (high-dose) mg/kg bw per day for 13 weeks. Animals were observed twice daily for clinical signs, mortality and behaviour.

No mortality or treatment-related clinical observations were noted throughout the study period. No significant effects were noted on body weight, food consumption, serum biochemistry or organ weights (heart, kidneys, liver, spleen, testes and thymus) in any group compared to the control group. Upon haematological analysis on day 46, a significant increase in granulocyte percentage in females of the mid-dose group and a significant decrease in glucose levels in males of the low-dose group were noted. The authors noted that these changes were not dose-related and were within the laboratory's normal control ranges. No significant changes were noted on any haematological parameter on day 91. A number of findings were noted upon histopathological examination, including sporadic focal necrosis

and spotty necrosis in liver, focal necrosis in heart, deposition of calcium salts in renal tubular in kidneys and testicular atrophy in testes. The incidence and severity of these changes were similar between the control group and animals administered the test material, and therefore were not considered treatment-related. Based on the results of this study, the authors determined the NOAEL of PQQ to be 400 mg/kg bw per day, in male and female rats, the highest dose tested. The Panel notes that urinary parameters were not investigated in this study.

The renal toxicity of the NF (BioPQQ™; purity > 99.1%) was investigated in a study wherein female Crl:CD(SD) rats (n = 12/sex per group) were administered the NF by gavage at doses of 0 (control), 200 (low dose) or 700 (high dose) mg/kg bw per day for 4 weeks (28 days), followed by a 4-week recovery period (n = 6/sex per group) (Mitsubishi Gas Chemical Company Inc, 2006b; Nakano et al., 2014). No mortality was reported, and there were no significant differences in body weight and water intake in any group, compared to the control group. Blackish stools were observed in the high-dose group during the administration period. This was suggested to be a result of the dark colour of the test material in the dosing formulations. Urinalysis revealed crystals (including ammonium magnesium phosphate, calcium oxalate and uric acid crystals) in urinary sediment of both female test groups (11/12 in low-dose and 12/12 in high-dose) at the end of the administration period. These effects were found to be alleviated by the end of the recovery period. Protein was detected in the urine of 1/12 controls, 5/12 low-dose and 6/12 high-dose females at the end of the administration period. At the end of the recovery period, this was still seen in one low- and two high-dose females. No histopathological lesions were observed in any group at the end of both the administration period and the recovery phase. The Panel notes the increased incidence of crystals and the presence of protein in urine at dose of 200 mg/kg bw and above. The Panel concludes that no NOAEL can be derived from this study.

In an intraperitoneal study, 11.5 mg PQQ/kg bw was administered for four consecutive days to five male Wistar rats (Watanabe et al., 1989). Five control rats were similarly injected with the vehicle. Rats were sacrificed 24 h after the last dose. Urinary excretion of protein, glucose, ketone body and occult blood was increased in the PQQ group, compared to the control group. Blood urea nitrogen and serum creatinine levels were significantly higher and serum triglyceride contents were significantly lower in the PQQ group, compared to the control group. Serum glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activities were higher in the PQQ group than in the control group. In the PQQ group, swelling of the kidneys was observed macroscopically at autopsy. Relative kidney weight was increased (PQQ: 0.91 $\pm$ 0.07% vs control: 0.65 $\pm$ 0.04%, $p < 0.001$). In the PQQ group, vacuolar degeneration, atrophy and necrosis of the proximal tubular epithelium in the renal cortex as well as dilatation and regeneration of the tubules were observed microscopically. A slight decrease in glycogen deposition and an accelerated mitotic process were observed also in the liver. The Panel notes that this study raises some concern regarding kidney toxicity but it should be taken into account that the PQQ was administered intraperitoneally. The Panel therefore considers that this study cannot be used in setting a NOAEL.

The Competent Authority of Ireland noted that enterohepatic cycling could explain the appearance of PQQ-related material in the faeces of rats in the toxicity studies. The Panel notes that the coloured faeces observed with doses between 100 and 768 mg PQQ/kg bw per day could be due to unabsorbed PQQ, which is a brown coloured compound with green fluorescence, and that no conclusion can be drawn from the available data on a possible enterohepatic cycling of the NF. The Panel considers that the darker-coloured faeces observed in these studies are not of toxicological concern.

The Panel considers the findings of crystal and protein in urine at 200 mg/kg bw in the 28-day toxicity study as the critical effect based on the fact that renal toxicity was observed at a concentration of 768 mg/kg bw in the 14-day study. Therefore, the Panel considers 100 mg/kg bw from the 90-day study which included a thorough urinalysis as the overall NOAEL.

3.7.5. Other animal studies

A few other studies in mice and broiler chicks involving oral administration of PQQ were provided by the applicant.

The effects of PQQ on reproductive performance were investigated in a study conducted by Steinberg et al. (1994) wherein female BALB/c mice were fed chemically defined diets containing up to 5,000 ng PQQ/g diet (approximately equivalent to 500 μg/kg bw per day) prior to mating, and during gestation and lactation. At weaning, the pups were fed the same diets as their respective dams for a

period of 20 weeks. No adverse effects were reported on reproduction, growth or lymphoid organ weights.

The same authors conducted a study to investigate the effects of PQQ on the growth, reproductive performance, and indices of collagen maturation and expression in mice (Steinberg et al., 2003). In this study, male and female BALB/c mice were fed chemically defined, amino acid-based diets with or without the addition of 6 μM of PQQ/kg diet (approximately equivalent to 220 μg/kg bw per day) for a period of 8 weeks prior to mating. The PQQ content in the basal amino acid diet was reported to be less than 5 fM/g diet. At weaning, pups were fed the same diets as their respective dams for a period of 35 days. No significant differences in relative liver, heart, kidney and spleen weights were reported among groups.

In a dry skin mouse model, mice (n = 8 per group) were administered 0.0089% w/w PQQ in the diet (providing ca. 16 mg/kg bw per day) for 6 weeks (Nakano et al., 2015a). Endpoints assessed were parameters of inflammation (skin thickness, mast cell number in dermis and CD^{3+} T-cell number in epidermis), transepidermal water loss (TEWL), skin conductance and food consumption (Nakano et al., 2015a). This study does not provide data of relevance for the safety assessment.

The effect of PQQ supplementation was evaluated in broiler chicks (Wang et al., 2015). Male 1-day-old Arbor Acres broiler chicks (7 replicates of 14 chicks/group) received up to 400 μg PQQ/kg feed (Shanghai Medical Life Sciences Research Center Co. Ltd, PQQ ≥ 99.5%), corresponding to approximately 40 μg/day for up to 42 days. During the course of the study, no differences in mortality rate were observed in any of the groups and no adverse health effects or differences in carcass quality reported.

The effects of dietary PQQ on growth performance, carcass yield and antioxidant status of broiler chicks was evaluated in a study by Samuel et al. (2015). One-day-old male Arbor Acres broilers (n = 784) were randomly allocated into one of seven dietary groups. The positive control/treatment groups were provided a diet containing antibiotics and various dietary levels of up to 800 μg PQQ/kg diet (Shanghai Medical Life Sciences Research Center Co. Ltd) for 42 days. Birds in the negative control group were fed a basal diet without antibiotics or PQQ. Administration of PQQ in the diet was not associated with adverse effects in the broilers.

The Panel considers that these studies were of short-term duration and were not specifically designed to assess toxicity and are therefore of limited value for the safety assessment.

3.7.6. Human studies

The applicant reported 12 clinical studies on PQQ.

Ten of these studies investigated some safety-related endpoints such as physical examination, clinical biochemistry and haematology parameters in blood, urinalysis and/or adverse effects reported by the subjects (Mitsubishi Gas Chemical Company Inc, 2006a, 2007, 2015a,b; Nakano et al., 2009, 2012, 2015b; Harris et al., 2013; Itoh et al., 2016; Krieger et al., 2016). Studies used PQQ doses ranging from 10 to 100 mg/day for study periods from 3 days to 24 weeks (N between 10 and 71). None of the studies showed significant effects of the study treatment on these outcomes.

In the two other studies, in which healthy adult subjects received 20 mg PQQ/day for up to 24 weeks, no adverse effects were reported by the authors (Koikeda et al., 2011; Nakano et al., 2015a).

No comprehensive evaluation of renal function was performed in any of these studies.

The Panel considers that these studies do not raise safety concern. The Panel notes, however, that these studies were not designed to assess renal function and are of limited value for the safety assessment.

3.7.7. Pro-oxidant activity of PQQ

A Member State raised concerns about the pro-oxidant properties of PQQ.

The applicant referred to three *in vitro* studies which investigated the anti-oxidant and pro-oxidant properties of PQQ (He et al., 2003; Ouchi et al., 2009; Ishii et al., 2010). Given the limited complexity of the *in vitro* experimental systems and the high concentrations applied (1 μM–1 mM) in these studies, the Panel considers that these studies are not representative of the *in vivo* situation at the proposed use levels of the NF.

Given the low plasma concentrations reached at the proposed use levels (i.e. nM) (Section 3.7.1), the Panel does not consider that there is a safety concern with respect to a pro-oxidant activity of the NF.

3.8. Allergenicity

No information has been provided regarding allergenicity.

However, considering the manufacturing process and composition of the NF, the Panel considers that the likelihood of allergic reactions to the NF is low.

4. Discussion

The NF is PQQ, which is an aromatic heterocyclic orthoquinone. The NF is produced by the bacterium strain *Hyphomicrobium denitrificans* CK-275 and the final form of the NF is a reddish-brown powder with a minimum purity of 99.0%.

The information provided on the composition, specifications, the production process, batch-to-batch variability and the stability of the NF is sufficient and does not raise safety concerns.

The applicant intends to market the NF for use in food supplements for healthy adults, except pregnant and lactating women. The maximum proposed level of consumption is 20 mg/day. Food supplements containing the NF are not intended for use by children.

PQQ occurs naturally in various food products. Considering the typical contents of PQQ in foods reported in the literature, the intake of PQQ in its free form from the background diet is at least 250 times lower than the proposed intake from the NF.

Based on the genotoxicity tests provided, the Panel concludes that there are no concerns regarding genotoxicity of the NF.

A 90-day repeated dose oral toxicity study with the NF was provided, which was administered to Sprague–Dawley rats at dosages of 3, 20 or 100 mg/kg bw per day of the NF. The Panel identified a NOAEL of 100 mg/kg bw per day, considering renal toxicity as the critical endpoint. The Panel notes that no chronic toxicity study was conducted.

Twelve clinical studies on PQQ with doses up to 100 mg and durations up to 24 weeks were provided. The Panel considers that these studies do not raise safety concern. The Panel notes, however, that these studies were not designed to assess renal function and are of limited value for the safety assessment.

Given the low plasma concentrations reached at the proposed use levels (i.e. nM), the Panel does not consider that there is a safety concern with respect to a pro-oxidant activity of the NF.

Considering the NOAEL of 100 mg/kg bw per day, and the maximum proposed level of consumption of 20 mg PQQ/day as food supplement (corresponding to 0.29 mg/kg bw per day for a 70-kg person), the Panel concludes that the margin of exposure (of 344) is sufficient.

Conclusions

The Panel concludes that the NF, pyrroloquinoline quinone disodium salt (BioPQQ™), is safe under the intended conditions of use as specified by the applicant.

Steps taken by EFSA

1) Letter from the European Commission to the European Food Safety Authority with the request for a scientific opinion on pyrroloquinoline quinone disodium salt as a novel food ingredient. Ref. Ares(2016)5904659, dated 13 October 2016.
2) Dossier 'Application for the Approval of Pyrroloquinoline Quinone Disodium Salt for Use in Food Supplements under Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 Concerning Novel Foods and Novel Food Ingredients', which was submitted by Mitsubishi Gas Chemical Company, Inc, received by EFSA on 13 October 2016.
3) On 18 November 2016, EFSA sent a request to the applicant to provide missing information to accompany the application.
4) On 16 December 2016, EFSA received the missing information as submitted by the applicant. After checking the content of the full dossier, including the missing information, EFSA considered the application valid as of 22 December 2016.
5) Initial assessment report carried out by the Food Safety Authority of Ireland: 'Safety Assessment of Pyrroloquinoline Quinone Disodium Salt (BioPQQ™)'.
6) Member States' comments and objections.
7) On 17 March 2017, EFSA sent a request to the applicant to provide additional information to accompany the application.
8) Additional data were provided by the applicant on 25 May 2017.

References

ABAS (Ausschuss für Biologische Arbeitsstoffe), 2015. Technische Regeln für Biologische Arbeitsstoffe. Einstufung von Prokaryonten (Bacteria und Archaea) in Risikogruppen. TRBA 466. 382 pp.

Anthony C and Zatman LJ, 1967. The microbial oxidation of methanol. The prosthetic group of the alcohol dehydrogenase of *Pseudomonas* sp. M27: a new oxidoreductase prosthetic group. Biochemical Journal, 104, 960–969.

Brown PJ, Kysela DT, Buechlein A, Hemmerich C and Brun YV, 2011. Genome sequences of eight morphologically diverse Alphaproteobacteria. Journal of Bacteriology, 193, 4567–4568.

Dekker RH, Duine JA, Frank J, Verwiel PE and Westerling J, 1982. Covalent addition of H_2O, enzyme substrates and activators to pyrrolo-quinoline quinone, the coenzyme of quinoproteins. European Journal of Biochemistry, 125, 69–73.

Duine JA and Jongejan JA, 1989. Quinoproteins, enzymes with pyrrolo-quinoline quinone as cofactor. Annual Review of Biochemistry, 58, 403–426.

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Ricci A, Allende A, Bolton D, Chemaly M, Davies R, Girones R, Koutsoumanis K, Lindqvist R, Nørrung B, Robertson L, Ru G, Fernandez Escamez P, Sanaa M, Simmons M, Skandamis P, Snary E, Speybroeck N, Ter Kuile B, Threlfall J, Wahlström H, Cocconcelli P, Peixe L, Maradona M, Querol A, Suarez J, Sundh I, Vlak J, Correia S and Herman L, 2017. Statement on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 6: suitability of taxonomic units notified to EFSA until March 2017. EFSA Journal 2017;15(7):4884, 32 pp. https://doi.org/10.2903/j.efsa.2017.4884

EFSA Scientific Committee, 2011. Scientific Opinion on genotoxicity testing strategies applicable to food and feed safety assessment. EFSA Journal 2011;9(9):2379, 69 pp. https://doi.org/10.2903/j.efsa.2011.2379

EFSA Scientific Committee, 2012. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 2012;10(3):2579, 32 pp. https://doi.org/10.2903/j.efsa.2012.2579

HAMAP, 2011. Hyphomicrobium denitrificans (strain ATCC 51888/DSM 1869/NCIB 11706/TK 0415) complete proteome. High-quality Automated and Manual Annotation of microbial Proteomes (HAMAP). Available online: http://hamap.expasy.org/proteomes/HYPDA.html

Harris CB, Chowanadisai W, Mishchuk DO, Satre MA, Slupsky CM and Rucker RB, 2013. Dietary pyrroloquinoline quinone (PQQ) alters indicators of inflammation and mitochondrial-related metabolism in human subjects. Journal of Nutritional Biochemistry, 24, 2076–2084.

Hauge JG, 1964. Glucose dehydrogenase of Bacterium anitratum: an enzyme with a novel prosthetic group. Journal of Biological Chemistry, 239, 3630–3639.

He K, Nukada H, Urakami T and Murphy MP, 2003. Antioxidant and pro-oxidant properties of pyrroloquinoline quinone (PQQ): implications for its function in biological systems. Biochemical Pharmacology, 65, 67–74.

Health Canada, 2016. Chemical Substance - Pyrroloquinoline quinone disodium salt. In: Natural Health Products Ingredients Database. Health Canada, Natural Health Products Directorate (NHPD). Available online: http://webprod.hc-sc.gc.ca/nhpid-bdipsn/ingredReq.do?id=12071&lang=eng

Houck DR, Hanners JL and Unkefer CJ, 1988. Biosynthesis of pyrroloquinoline quinone. 1. Identification of biosynthetic precursors using carbon-13 labeling and NMR spectroscopy. Journal of the American Chemical Society, 110, 6920–6921.

Ishii T, Akagawa M, Naito Y, Handa O, Takagi T, Mori T, Kumazawa S, Yoshikawa T and Nakayama T, 2010. Pro-oxidant action of pyrroloquinoline quinone: characterization of protein oxidative modifications. Bioscience, Biotechnology, and Biochemistry, 74, 663–666.

Itoh Y, Hine K, Miura H, Uetake T, Nakano M, Takemura N and Sakatani K, 2016. Effect of the antioxidant supplement pyrroloquinoline quinone disodium salt (BioPQQ) on cognitive functions. Advances in Experimental Medicine and Biology, 876, 319–325.

Kikuchi M, 2011. Safety study report: pathogenicity study [PQQ-producing CK-275 Strain]. Study report no. PT-1105. Mitsubishi Gas Chemical Company, unpublished confidential study report, 22 pp.

Kikuchi M, 2012. Safety study report: acute oral toxicity study [PQQ-producing CK-275 Strain]. Study report no. ATT-1207. Mitsubishi Gas Chemical Company, unpublished confidential study report, 16 pp.

van Kleef MAG and Duine JA, 1988. L-tyrosine is the precursor of PQQ biosynthesis in Hyphomicrobium X. FEBS Letters, 237, 91–97.

Koikeda T, Nakano M and Masuda K, 2011. Pyrroloquinoline quinone disodium salt improves higher brain function. Shinryo To Shinyaku [Medical Consultation & New Remedies], 48, 519–527.

Krieger D, Kalman D, Feldman S, Samson A and Nader S, 2016. The safety of pyrroloquinoline quinone disodium in older adults with self-reported memory loss. QPS MRA (Miami Research Associates). Confidential manuscript 12 pp.

Kumazawa T, Seno H, Urakami T, Matsumoto T and Suzuki O, 1992. Trace levels of pyrroloquinoline quinone in human and rat samples detected by gas chromatography/mass spectrometry. Biochimica et Biophysica Acta: Protein Structure and Molecular Enzymology, 1156, 62–66.

Kumazawa T, Seno H and Suzuki O, 1993. Failure to verify high levels of pyrroloquinoline quinone in eggs and skim milk. Biochemical and Biophysical Research Communications, 193, 1–5.
Kumazawa T, Sato K, Seno H, Ishii A and Suzuki O, 1995. Levels of pyrroloquinoline quinone in various foods. Biochemical Journal, 307(Pt. 2), 331–333.
Liang C, Zhang X, Wang W, Song Y and Jia X, 2015. A subchronic oral toxicity study on pyrroloquinoline quinone (PQQ) disodium salt in rats. Food and Chemical Toxicology, 75, 146–150.
Mitchell AE, Jones AD, Mercer RS and Rucker RB, 1999. Characterization of pyrroloquinoline quinone amino acid derivatives by electrospray ionization mass spectrometry and detection in human milk. Analytical Biochemistry, 269, 317–325.
Mitsubishi Gas Chemical Company Inc, 2005a. A 14-day dose finding study of pyrroloquinoline quinone for a 90-day repeated oral dose toxicity study in rats. Study no. 05165. Unpublished study report, 192 pp.
Mitsubishi Gas Chemical Company Inc, 2005b. Mutagenicity study of pyrroloquinoline quinone (PQQ) with the bacterial reverse mutation assay. Study Number 10469. Unpublished study report, 17 pp.
Mitsubishi Gas Chemical Company Inc, 2006a. A 4-week intake study of pyrroloquinolinequinone disodium salt (PQQ) in healthy volunteers. Clinical study report - Study no. 06151. Unpublished confidential study report, 30 pp.
Mitsubishi Gas Chemical Company Inc, 2006b. Renal toxicity study of PQQ in rats with a recovery period. Study No. DA06093. Unpublished study report, 55 pp.
Mitsubishi Gas Chemical Company Inc, 2006c. A micronucleus test of PQQ in mice. Study No. DA06164. Unpublished study report, 23 pp.
Mitsubishi Gas Chemical Company Inc, 2006d. A chromosomal aberration test of PQQ in cultured mammalian cells. Study No. DA06235. Unpublished study report, 25 pp.
Mitsubishi Gas Chemical Company Inc, 2007. Double-blind controlled safety evaluation of excessively ingested pyrroloquinoline quinone disodium salt (PQQ): Human food ingestion study report. Study Protocol Number: MCM-0702-MGC. Unpublished confidential study report, 18 pp.
Mitsubishi Gas Chemical Company Inc, 2008a. A chromosomal aberration test of PQQ in cultured mammalian cells (2). Study No. DA07437. Unpublished study report, 26 pp.
Mitsubishi Gas Chemical Company Inc, 2008b. In vitro chromosomal aberration test of pyrroloquinoline quinone disodium salt with human peripheral blood lymphocytes. Study No. DA08023. Unpublished study report, 28 pp.
Mitsubishi Gas Chemical Company Inc, 2015a. Efficacy test of pyrroloquinoline quinone for brain function by near infrared time resolved spectroscopy (Second test). Results report. Unpublished study report, 36 pp.
Mitsubishi Gas Chemical Company Inc, 2015b. Safety confirmation owing to excessive intake of pyrroloquinoline quinone (PQQ) (Second test). Results report. Unpublished study report, 32 pp.
Nakano M, Ubukata K, Yamamoto T and Yamaguchi H, 2009. Effect of pyrroloquinoline quinone (PQQ) on mental status of middle-aged and elderly persons. Food Style, 21, 50–53.
Nakano M, Yamamoto T, Okamura H, Tsuda A and Kowatari, Y, 2012. Effects of oral supplementation with pyrroloquinoline quinone on stress, fatigue, and sleep. Functional Foods in Health and Disease, 2, 307–324.
Nakano M, Suzuki H, Imamura T, Lau A and Lynch B, 2013. Genotoxicity of pyrroloquinoline quinone (PQQ) disodium salt (BioPQQ). Regulatory Toxicology and Pharmacology, 67, 189–197.
Nakano M, Takahashi H, Koura S, Chung C, Tafazoli S and Roberts A, 2014. Acute and subchronic toxicity studies of pyrroloquinoline quinone (PQQ) disodium salt (BioPQQ) in rats. Regulatory Toxicology and Pharmacology, 70, 107–121.
Nakano M, Kamimura A, Watanabe F, Kamiya T, Watanabe D, Yamamoto E, Fukagawa M, Hasumi K and Suzuki E, 2015a. Effects of orally administered pyrroloquinoline quinone disodium salt on dry skin conditions in mice and healthy female subjects. Journal of Nutritional Science and Vitaminology (Tokyo), 61, 241–246.
Nakano M, Kawasaki Y, Suzuki N and Takara T, 2015b. Effects of pyrroloquinoline quinone disodium salt intake on the serum cholesterol levels of healthy Japanese adults. Journal of Nutritional Sciences and Vitaminology (Tokyo), 61, 233–240.
Noji N, Nakamura T, Kitahata N, Taguchi K, Kudo T, Yoshida S, Tsujimoto M, Sugiyama T and Asami T, 2007. Simple and sensitive method for pyrroloquinoline quinone (PQQ) analysis in various foods using liquid chromatography/electrospray-ionization tandem mass spectrometry. Journal of Agricultural and Food Chemistry, 55, 7258–7263.
OECD (Organisation for Economic Cooperation and Development), 1998. Test No. 408: repeated dose 90-day oral toxicity study in rodents. 10 pp.
OECD (Organisation for Economic Cooperation and Development), 2011. Test No. 423: acute oral toxicity – acute toxic class method. 14 pp.
Ouchi A, Nakano M, Nagaoka S and Mukai K, 2009. Kinetic study of the antioxidant activity of pyrroloquinolinequinol (PQQH(2), a reduced form of pyrroloquinolinequinone) in micellar solution. Journal of Agricultural and Food Chemistry, 57, 450–456.
Rucker R, Chowanadisai W and Nakano M, 2009. Potential physiological importance of pyrroloquinoline quinone. Alternative Medicine Review, 14, 268–277.
Salisbury SA, Forrest HS, Cruse WB and Kennard O, 1979. A novel coenzyme from bacterial primary alcohol dehydrogenases. Nature, 280, 843–844.

Samuel KG, Zhang HJ, Wang J, Wu SG, Yue HY, Sun LL and Qi GH, 2015. Effects of dietary pyrroloquinoline quinone disodium on growth performance, carcass yield and antioxidant status of broiler chicks. Animal, 9, 409–416.
Smidt CR, Unkefer CJ, Houck DR and Rucker RB, 1991. Intestinal absorption and tissue distribution of [14C] pyrroloquinoline quinone in mice. Proceedings of the Society for Experimental Biololgy and Medicine, 197, 27–31.
Steinberg FM, Gershwin ME and Rucker RB, 1994. Dietary pyrroloquinoline quinone: growth and immune response in BALB/c mice. Journal of Nutrition, 124, 744–753.
Steinberg F, Stites TE, Anderson P, Storms D, Chan I, Eghbali S and Rucker R, 2003. Pyrroloquinoline quinone improves growth and reproductive performance in mice fed chemically defined diets. Experimental Biology and Medicine (Maywood), 228, 160–166.
Stites TE, Mitchell AE and Rucker RB, 2000. Physiological importance of quinoenzymes and the O-quinone family of cofactors. Journal of Nutrition, 130, 719–727.
Urakami T, Yashima K, Kobayashi H, Yoshida A and Ito-Yoshida C, 1992. Production of pyrroloquinoline quinone by using methanol-utilizing bacteria. Applied and Environmental Microbiology, 58, 3970–3976.
Urakami T, Sasaki J, Suzuki K-I and Komagata K, 1995. Characterization and description of *Hyphomicrobium denitrificans* sp. nov. International Journal of Systematic and Evolutionary Microbiology, 45, 528–532.
US FDA (Food and Drug Administration), 2007. Subchronic toxicity studies with rodents. In: Guidance for industry and other stakeholders. Toxicological principles for the safety assessment of food ingredients. Redbook 2000.
Wang L-Y, Zhao R, Shi W-Q and Cheng G, 2012. Toxicological safety assessment of the pyrroloquinoline quinine. Jiangsu Journal of Preventive Medicine, 23, 22–24.
Wang J, Zhang HJ, Samuel KG, Long C, Wu SG, Yue HY, Sun LL and Qi GH, 2015. Effects of dietary pyrroloquinoline quinone disodium on growth, carcass characteristics, redox status, and mitochondria metabolism in broilers. Poultry Science, 94, 215–225.
Watanabe A, Hobara N, Ohsawa T, Higashi T and Tsuji T, 1989. Nephrotoxicity of pyrroloquinoline quinone in rats. Hiroshima Journal of Medical Sciences, 38, 49–51.

Abbreviations

ADME	absorption, distribution, metabolism and excretion
ATCC	American type culture collection
bw	body weight
CAS	Chemical Abstracts Service
CFU	colony forming unit
cGMP	current Good Manufacturing Practices
DSMZ	German collection of microorganisms and cell cultures
FDA	Food and Drug Administration
GLP	good laboratory practice
GOT	glutamate oxaloacetate transaminase
GPT	glutamate pyruvate transaminase
HACCP	Hazard Analysis Critical Control Points
HPLC	high-performance liquid chromatography
IPQ	imidazolopyrroloquinoline quinone
ISO	International Organization for Standardization
JFSL	Japan Food Sanitation Law
LD_{50}	median lethal dose
MS	mass spectrometry
NCIB	national collection of industrial bacteria
NDA	EFSA Panel on Dietetic Products, Nutrition and Allergies
NF	novel food
NOAEL	no-observed-adverse-effect-level
OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
PQQ	pyrroloquinoline quinone disodium salt
QPS	qualified presumption of safety
SD	standard deviation
TEWL	transepidermal water loss
UV	ultraviolet