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Safety of a botanical extract derived from *Panax notoginseng* and *Astragalus membranaceus* (AstraGin™) as a novel food pursuant to Regulation (EU) 2015/2283

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Abstract

Following a request from the European Commission, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver a scientific opinion on a botanical extract derived from both *Panax notoginseng* and *Astragalus membranaceus* (AstraGin™) as a novel food (NF) pursuant to Regulation (EU) 2015/2283. The NF is a combination of an ethanol extract of the roots of *A. membranaceus* and a hot water extract of the roots of *P. notoginseng*. The NF contains 1.5–5% total saponins, 0.1–0.5% ginsenoside Rb1 and 0.01–0.1% astragaloside I. Both plants that are used to produce the NF have a long history of use, especially in traditional Chinese medicine. Information on the production process and the composition of the NF is sufficient and does not raise safety concerns. The applicant proposed to use the NF as a food supplement for the general adult population, excluding pregnant women, at a maximum daily amount of 350 mg. Taking into account these conditions of use, the Panel considers that the consumption of the NF is not nutritionally disadvantageous. The provided genotoxicity studies do not raise concerns for genotoxicity of the NF. Based on the findings of a subchronic toxicity study, supported by a subacute toxicity study, the Panel identified the overall no observed adverse effect level (NOAEL) of the NF at 100 mg/kg body weight (bw) per day. By applying an uncertainty factor of 200, the Panel concludes that the NF is safe at an intake level of 0.5 mg/kg bw per day, corresponding to a maximum daily intake of 35 mg of the NF for the target population, i.e. adults excluding pregnant women.

Keywords: *Panax notoginseng*, *Astragalus membranaceus*, *Astragalus propinquus*, extract, novel food, safety

Requestor: European Commission following an application by NuLiv Science

Question number: EFSA-Q-2018-00557

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1. Introduction

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

On 8 June 2018, the company NuLiv Science submitted a request to the European Commission in accordance with Article 10 of Regulation (EU) 2015/2283[1] to place a botanical extract derived from both *Panax notoginseng* and *Astragalus membranaceus* (AstraGin™) on the Union market as a novel food (NF).

The NF is intended for use in food supplements and the suggested daily intake for adults is 50 mg.

The target population is the general population, excluding children and pregnant women.

On 22 October 2018, and in accordance with Article 10(3) of Regulation (EU) 2015/2283, the European Commission asked EFSA to provide a scientific opinion on the extract derived from *Panax notoginseng* and *Astragalus membranaceus*.

2. Data and methodologies

2.1. Data

The safety assessment of this NF is based on data supplied in the application, information provided by the EFSA Working Group on Compendium of Botanicals and information submitted by the applicant following two EFSA requests for supplementary information.

The information in support of this application has been made available through the Commission e-submission portal (NF 2018/0284).

Administrative and scientific requirements for NF applications referred to in Article 10 of Regulation (EU) 2015/2283 are listed in the Commission Implementing Regulation (EU) 2017/2469[2].

A common and structured format on the presentation of NF applications is described in the EFSA guidance on the preparation and presentation of a NF application (EFSA NDA Panel, 2016). As indicated in this guidance, it is the duty of the applicant to provide all of the available (proprietary, confidential and published) scientific data, including both data in favour and not in favour of supporting the safety of the proposed NF.

This NF application includes a request for protection of proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283. Data claimed to be proprietary by the applicant include all the submitted analytical information, the manufacturing process and the toxicity studies provided.

2.2. Methodologies

The assessment follows the methodology set out in the EFSA guidance on NF applications (EFSA NDA Panel, 2016) and the principles described in the relevant existing guidance documents from the EFSA Scientific Committee. The legal provisions for the assessment are laid down in Article 11 of Regulation (EU) 2015/2283 and in Article 7 of the Commission Implementing Regulation (EU) 2017/2469.

The information provided by the EFSA Working Group on Compendium of Botanicals is based on extensive literature searches on *P. notoginseng* and *A. membranaceus*, following a search strategy and standard operating procedure as described by the University of Chemistry and Technology (UCT) of Prague (UCT, 2020).

This assessment concerns only risks that might be associated with consumption of the NF under the proposed conditions of use, and is not an assessment of the efficacy of AstraGin™ with regard to any (claimed) benefit.

3. Assessment

3.1. Introduction

The NF which is the subject of the application is a combination of root extracts from *P. notoginseng* and *A. membranaceus*, denominated as AstraGin™.

[1] Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001 (2013/0435 (COD). OJ L 327, 11.12.2015, p. 1–22.

[2] Commission Implementing Regulation (EU) 2017/2469 of 20 December 2017 laying down administrative and scientific requirements for applications referred to in Article 10 of Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. OJ L 351, 30.12.2017, pp. 64–71.

The NF falls under Regulation 2015/2283, Article 3(2)(a)(iv): food consisting of, isolated from or produced from plants or their parts of.

The NF is proposed by the applicant to be used as a food supplement for the general adult population, except pregnant women.

3.2. Identity of the NF

The NF is a combination of an ethanol extract of the roots of *A. membranaceus* (Fisch.) Bunge and a hot water extract of the roots of *P. notoginseng* (Burkill) F.H. Chen.

3.2.1. Panax notoginseng (Burkill) F.H. Chen

The full taxonomy of *P. notoginseng* is the following. Kingdom: Plantae, Division: Magnoliophyta, Class: Magnoliopsida, Order: Apiales, Family: Araliaceae, Genus: *Panax*, Species: *Panax notoginseng*.

The plant is also known as *Panax pseudoginseng* var. *notoginseng* (Burkill) G. Hoo & C.L. Tseng and by a number of traditional Chinese names, e.g. Sanqi, San Qi, Shan Qi, Tu San Qi, Tianqi and Tien-chi.

The plant belongs to the genus *Panax*, which includes about 15 species from both Asia and America. Analyses of the 5S-rRNA spacer domains from seven Panax species showed a high degree of genetic similarity with ~ 75% of the spacer domains conserved throughout the genus (Cui et al., 2003).

The root of *P. notoginseng* contains ~ 8–13% saponins by weight (Dong et al., 2003; Wang et al., 2006). More than 150 saponins, mostly of dammarane type with 20(S)-protopanaxadiol or 20(*S*)-protopanaxatriol aglycon moieties have been identified in this species, some of them being present in other *Panax* species, some being specific of *P. notoginseng*. Many other compounds of various chemical classes are found including flavonoids, cyclopeptides, sterols, polyacetylenes, a volatile oil, saccharides and amino acids (Wang et al., 2016). Based on an earlier review, the applicant stated that 56 saponins of *P. notoginseng* have been identified with major ones being ginsenosides Rb1, Rd, Re and Rg1. Five polyacetylenes have been identified, i.e. panaxytriol, panaxydol, notoginsenic acid, β-sophoroside and 10-hydroxydeca-4,6-diynoic acid. The extract also contained the flavonoids quercetin and quercetin-3-*O*-sophoroside, a number of phytosterols and amino acids, the most prevalent ones being arginine, aspartic acid and glutamic acid. The non-proteinogenic amino acid L-dencichin (oxalyldiaminopropionic acid) has also been identified (Wang et al., 2006).

3.2.2. *Astragalus membranaceus* (Fisch.) Bunge

The name *A. membranaceus* (Fisch.) Bunge is a synonym of the accepted name *Astragalus propinquus* Schischkin.[3]

The full taxonomy of *A. membranaceus/A. propinquus* is the following. Kingdom: Plantae, Division: Magnoliophyta, Class: Magnoliopsida, Order: Fabales, Family: Fabaceae, Genus: *Astragalus*, Species: *A. membranaceus*/*A. propinquus*.

The plant is also known as milkvetch root (English), ogi (Japanese), hwanggi (Korean) and huang qi (in Traditional Chinese Medicine).

This species is defined as Radix Astragali in the Pharmacopoeia of China (Fu et al., 2014).

The root of *A. membranaceus/A. propinquus* contains a number of saponins, in particular cycloartane triterpene glycosides (astragalosides I–VII) as well as flavonoids (calycosin and formononetin genins and glycosides, ononin, among others). Other constituents include γ- and α-aminobutyric acids, L-canavanine, choline, betaine, gluconic acid, linoleic acid, asparagine, β-sitosterol (Thorne Research Inc, 2003; Fu et al., 2014).

3.3. Production process

According to the information provided, the NF is produced under adherence to good manufacturing practice (GMP) at GMP-certified manufacturing facilities in China. Information was provided on the Hazard Analysis and Critical Control Points (HACCP) scheme applied.

The *A. membranaceus* roots are purchased from growers in Gansu and Inner Mongolia provinces in the north-western region of China while the *P. notoginseng* roots are acquired from growers in Yunnan and Sichuan provinces in the southern region of China.

[3] http://www.theplantlist.org/tpl1.1/record/ild-32156

The roots of both plants are washed with water, dried and pulverised.

The pulverised roots of *A. membranaceus* are then passed through a 10-mesh size sieve and extracted with eightfold volume of 40% ethanol. The extract is centrifuged, filtered, concentrated/dried, smashed and screened through an 80-mesh size sieve. The extract is analysed for the content of total saponins (by ultraviolet (UV) spectrophotometry) and for astragaloside I (by high-performance liquid chromatography-evaporative light scattering detection (HPLC-ELSD)).

The pulverised roots of *P. notoginseng* are passed through a 20-mesh size sieve and extracted with 10-fold volume of water. The extract is centrifuged, filtered and concentrated. The concentrated solution is subsequently absorbed on a resin, which is washed with water, and eluted with 60% ethanol. The eluent is concentrated/dried, smashed and screened through an 80-mesh size sieve. The extract is analysed for the content of total saponins (by UV spectrophotometry) and for ginsenoside Rb1 (by HPLC-UV).

The plant to extract ratios were indicated by the applicant to be 10:1 for the *Astragalus* extract and 50:1 for the *Panax* extract.

At the end of the manufacturing process the extracts are mixed (45–47.5% of each extract) with maltodextrin (5–10%). The blend, i.e. the NF, is analysed for the marker compounds (total saponins, ginsenoside Rb1 and astragaloside I; see the following Section), physicochemical characteristics, heavy metals, pesticides, organic solvent residue and microbial counts before packaging into plastic bags for storage.

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

3.4. Compositional data

As described in the previous section, the NF is a blend of an ethanol extract of the roots of *A. membranaceus* and a hot water extract of the roots of *P. notoginseng* (plus maltodextrin as an excipient). The NF contains 1.5–5% total saponins, 0.1–0.5% ginsenoside Rb1 and 0.01–0.1% astragaloside I.

In order to provide information on the variability of the composition of the NF, the applicant submitted batch to batch analyses for total saponins, ginsenoside Rb1, astragaloside I, proximates, a number of organoleptic and physicochemical parameters, contaminants and microbiological quality of the NF (Table 1).

Table 1: Batch-to-batch analyses of the NF

Parameter	Batch number				
	C20151209	**C20160606(1)**	**C20170106**	**C20170515**	**C20171009**
Total saponins	4.10%	3.79%	3.12%	3.56%	3.09%
Ginsenoside Rb1	0.10%	0.17%	0.14%	0.11%	0.10%
Astragaloside I	0.034%	0.028%	0.028%	0.024%	0.048%
Colour	Light yellow	Light yellow	Light yellow	Light yellow	Light yellow
Odour	'Characteristic'	'Characteristic'	'Characteristic'	'Characteristic'	'Characteristic'
Appearance	Fine powder	Fine powder	Fine powder	Fine powder	Fine powder
Sieve analysis	100% through 80 mesh	99% through 80 mesh	100% through 80 mesh	100% through 80 mesh	100% through 80 mesh
Total carbohydrates	91.8%	92.2%	92.1%	92.4%	89.6%
Protein	2.1%	2.5%	2.4%	2.5%	4.3%
Fat	1.4%	0.8%	0.7%	0.7%	1.1%
Moisture	4.3%	4.1%	4.5%	4.2%	4.6%
Ash	0.4%	0.4%	0.3%	0.3%	0.4%
Bulk density	0.59 g/mL	0.54 g/mL	0.56 g/mL	0.55 g/mL	0.53 g/mL
Solubility in water (1 g in 30 mL)	Soluble	Soluble	Soluble	Soluble	Soluble
Contaminants					
Lead (Pb)	< 0.1 mg/kg	< 0.1 mg/kg	< 0.1 mg/kg	< 0.1 mg/kg	< 0.1 mg/kg
Arsenic (As)	0.19 mg/kg	< 0.1 mg/kg	< 0.1 mg/kg	< 0.1 mg/kg	< 0.1 mg/kg

Parameter	Batch number				
	C20151209	C20160606(1)	C20170106	C20170515	C20171009
Cadmium (Cd)	< 0.01 mg/kg	< 0.01 mg/kg	< 0.01 mg/kg	< 0.01 mg/kg	< 0.01 mg/kg
Mercury (Hg)	< 0.01 mg/kg	< 0.01 mg/kg	< 0.01 mg/kg	0.02 mg/kg	0.02 mg/kg
Ethanol	< 0.5%	< 0.5%	< 0.5%	< 0.5%	< 0.5%
Microbiological					
Total plate count	370 CFU/g	35 CFU/g	410 CFU/g	900 CFU/g	< 10 CFU/g
Yeast and mould	210 CFU/g	20 CFU/g	60 CFU/g	< 20 CFU/g	< 20 CFU/g
Enterobacteriaceae	–	< 10 CFU/g	< 10 CFU/g	< 10 CFU/g	< 10 CFU/g
E. coli (in 25 g)	Negative	Negative	Negative	Negative	Negative
Salmonella (in 375 g)	Negative	Negative	Negative	Negative	Negative
Staphylococcus aureus (in 25 g)	Negative	Negative	Negative	Negative	Negative

NF: novel food; CFU: colony forming units.
(1): Batch of the NF that was used in the 90-day toxicity study (see Section 3.10.2).

For four additional batches (Table 2) information on the content of total saponins was provided, plus for three of the four batches also the concentrations of astragaloside I and ginsenoside Rb1.

Table 2: Total saponins, astragaloside I and ginsenoside Rb1 in the NF

Parameter	Batch number			
	20100621(1)	C20150120(2)	20100824	C20140921
Total saponins	2.35%	2.68%	2.92%	2.0%
Ginsenoside Rb1	0.17%	0.10%	0.10%	Not provided
Astragaloside I	0.054%	0.033%	0.039%	Not provided

NF: novel food.
(1): Batch of the NF that was used in the 28-day toxicity study.
(2): Batch of the NF that was used in the bacterial reverse mutation test and in the *in vitro* mammalian cell gene mutation assay (see Section 3.10.1).

In order to achieve a certain concentration of total saponins and ginsenoside Rb1 and astragaloside I in the NF, the applicant established internal specifications for the two extracts, with total saponins ≥ 0.6% and ginsenoside Rb1 ≥ 0.2% for the *P. notoginseng* extract, and total saponins ≥ 3% and astragaloside I ≥ 0.02% for the *A. membranaceus* extract.

Considering that the genus *Astragalus* is known to accumulate trace elements, the applicant provided a certificate of analysis for one batch of the NF, in which selenium, thallium and uranium were below detection limits (i.e. < 0.1 mg/kg for selenium and thallium and < 0.01 mg/kg for uranium).

Additionally, the applicant provided the analytical results for the concentrations of pesticides and mycotoxins in the NF, which were all below their limits of detection (LODs).

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

3.4.1. Stability

The applicant proposes a shelf-life of 3 years from the time of manufacturing of the NF.

The applicant provided data of a 6 months accelerated (i.e. at 40°C and 75% relative humidity) stability testing performed with three batches of the NF. The batches were analysed for the content of total saponins in the NF, which remained stable throughout the testing period (Table 3). Further parameters were appearance (i.e. light yellow powder), moisture, ash, heavy metals and microbial counts, which all remained within specifications during the testing period.

Table 3: Total saponin content of the NF during the stability testing

Date of analysis	Batch number		
	20100621	20100720	20100825
22/10/2010	2.35%	2.65%	2.92%
22/11/2010	2.39%	2.68%	2.84%
22/12/2010	2.37%	2.59%	2.88%
22/1/2011	2.41%	2.61%	2.86%
22/4/2011	2.43%	2.61%	2.93%

NF: novel food.

The Panel considers that, given the low moisture content of the NF, eventual degradation products or microbial activity are unlikely to be of safety concern. Since the product parameters were unchanged for six months under accelerated conditions the product can be expected to be stable for up to 2 years.

3.5. Specifications

The specifications of the NF are indicated in Table 4.

The applicant informed that each batch of the NF will be subjected to a series of analytical quality controls to ensure that all product specifications are met, including physicochemical parameters, organoleptic characteristics, microbial counts, heavy metals and pesticide residues.

Table 4: Specifications of the NF

Description: The NF is a combination of an ethanol extract of the roots of *Astragalus membranaceus* (Fisch.) Bunge and a hot water extract of the roots of *Panax notoginseng* (Burkill) F.H. Chen Appearance: beige to light brown yellow powder		
Parameter	**Specification**	**Method of analysis**
Total saponins	1.5–5.0%	UV absorption (at 560 ± 5 nm)
Ginsenoside Rb1	0.10–0.50%	HPLC-UV
Astragaloside I	0.01–0.10%	HPLC-ELSD
Carbohydrates	≥ 90%	By calculation
Protein	≤ 4.5%	Kjeldahl
Moisture	≤ 5.0%	GB/T 14769 -1993
Ash	≤ 1.0%	AOAC 942.05, 18th
Contaminants		
Arsenic (As)	≤ 0.3 mg/kg	ChP 2015, App. IXB (ICP-MS)
Microbiological		
Total plate count	≤ 5,000 CFU/g	ISO 4833-1
Total yeast and mould count	≤ 500 CFU/g	FDA (BAM) Chapter 18, 8th Ed.
Enterobacteriaceae	< 10 CFU/g	ISO 21528-2:2004
E. coli	Negative in 25 g	ISO 7251:2005
Salmonella	Negative in 375 g	FDA (BAM) Chapter 5, 8th Ed.
Staphylococcus aureus	Negative in 25 g	GB 5009.22-2016

CFU: colony forming units; FDA-BAM: Food and Drug Administration's Bacteriological Analytical Manual; GC–MS: gas chromatography–mass spectrometry; HPLC: high-performance liquid chromatography; ICP-MS:inductively coupled plasma mass spectrometry; UV: ultraviolet; ELSD: evaporative light scattering detector.

The Panel considers that the information provided on the specifications of the NF is sufficient and does not raise safety concerns.

3.6. History of use of the NF and/or of its source

3.6.1. History of use of the source of the NF

Both plants *A. membranaceus* and *P. notoginseng* have a long history of use in humans, especially in China.

According to the information provided, *A. membranaceus* has a recorded history of use of over 2,000 years (Khan and Abourashed, 2009). Traditionally, it has mainly been used as raw dried root, honey-cured root or as aqueous decoction, although steeping in ethanol has also been described (Reid, 1995; Khan and Abourashed, 2009). Daily doses of the root are reported to range from 9 to 30 g, with more than 60 g in some cases (Duke and Ayensu, 1985; Leung, 1995; Leung and Foster, 1996; Zhu, 1998b; Yu et al., 2007).

Documented use of *P. notoginseng* dates back to the 16th century and its cultivation in certain areas has been reported to exceed 1,000 years, with current production in China of approximately 2,000 tonnes per year (Dong et al., 2003). Typical total daily doses range from approximately 3 to 15 g of roots for a decoction or 1–5 g of root as a ground powder. The use of ethanolic extracts was also reported (Bensky and Gamble, 1986; Zhu, 1998a; Dharmananda, 2004; Wang et al., 2006; Reid, 1987).

The Panel notes that extensive literature searches performed by UCT Prague retrieved 13,581 and 8,116 scientific articles for *A. membranaceus* and *P. notoginseng*, respectively, demonstrating that these species have been extensively studied.

3.6.2. History of use of the NF

According to the information provided by the applicant, the NF is already marketed as a food supplement in a number of countries worldwide (e.g. Australia, Brazil, Canada, India).

Sales data have been provided for individual countries for the years 2011, 2012 and 2013 (confidential information).

3.7. Proposed uses and use levels

3.7.1. Target population

The target population proposed by the applicant is the general adult population, except pregnant women.

3.7.2. Proposed uses and use levels

The applicant proposed to use the NF as a food supplement.

Following a request for clarification, the applicant proposed an amount of 50 mg per portion and a maximum daily amount of 350 mg of the NF.

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

The applicant has not provided any toxicokinetic studies that were performed with the NF.

A number of animal studies were submitted, which investigated the absorption, distribution, metabolism and excretion of various saponins. The Panel considers that the studies provided are of limited relevance for the safety assessment of the NF.

3.9. Nutritional information

The applicant provided a nutritional analysis of the NF, which indicates that the NF mainly consists of carbohydrates (about 90%), moisture (about 5%), protein (2–5%), fat (about 1%) and ash. The applicant pointed out that some variation can be expected concerning the nutrients in the NF, given the nature of the starting material and its susceptibility to varying weather conditions.

Taking into account the proposed conditions of use (i.e. max. 350 mg/day), the Panel considers that the consumption of the NF is not nutritionally disadvantageous.

3.10. Toxicological information

The roots of *A. membranaceus* have been reported to contain the non-proteinogenic amino acid L-canavanine (Thorne Research Inc, 2003; Khan and Abourashed, 2009). L-Canavanine is an antinutrient that is structurally related to L-arginine. Owing to this similarity, in organisms that consume L-canavanine, it is mistakenly incorporated into their own proteins in place of L-arginine, leading to the production of structurally aberrant proteins. L-Canavanine has been suspected of being associated with systemic lupus erythematosus (SLE) activation (Alcocer-Varela et al., 1985; Morimoto et al., 1990). L-Canavanine is found in many legumes where it acts as a nitrogen-storing metabolite and is part of the plant's chemical defence. For example, L-canavanine accounts for up to 5% of the dry weight in the jack bean seed (*Canavalia ensiformis*), 2.4% in the alfalfa plant (*Medicago sativa*) and up to 2.5% in sword bean (*Canavalia gladiate*) (Rosenthal, 2001; Bence and Crooks, 2003). Following an EFSA request to provide information on the content of L-canavanine in the NF, the applicant submitted a certificate of analysis for one batch of the NF in which L-canavanine was detected at a concentration of 0.67 mg/g. The Panel notes that the amount of L-canavanine at the proposed conditions of use for the NF (i.e. 350 mg per day) would be 0.23 mg/day. The Panel considers L-canavanine in the NF unlikely to be of safety concern at the proposed conditions of use.

P. notoginseng has been reported to contain the non-proteinogenic amino acid L-dencichin (oxalyldiaminopropionic acid (ODAP)) (Wang et al., 2006), which is a structural analogue of the neurotransmitter glutamate. L-Dencichin is a neurotoxin responsible for lathyrism, a motor neuron degeneration syndrome. Lathyrism may be induced by consumption of the seeds (if consumed at high enough amounts) of the legume *Lathyrus sativus* (grass pea), which is a staple food in some areas of the world and which has been reported to contain L-dencichin at concentrations up to 0.7% (Xu et al., 2017). Following an EFSA request to provide information on the content of L-dencichin in the NF, the applicant submitted a certificate of analysis for one batch of the NF in which L-dencichin was not detected. No LOD was provided. However, information was provided on the method that was used for the analysis (Dezhuang et al., 1998). From the information provided, the limit of quantification (LOQ) for L-dencichin is at a level of about 0.17% for the method used. Taking into consideration the maximum proposed use level of 350 mg NF/day, and assuming an L-dencichin concentration at the LOQ (i.e. 0.17%) in the *P. notoginseng* fraction (i.e. < 50% in the NF), this would correspond to about 0.3 mg L-dencichin/day. The Panel considers L-dencichin in the NF unlikely to be of safety concern at the proposed conditions of use.

3.10.1. Genotoxicity

The applicant provided a bacterial reverse mutation assay (Zin, 2016, unpublished study report) and an *in vitro* mammalian cell gene mutation assay (Ruslan et al., 2016, unpublished study report), which were both performed with batch #C20150120 of the NF. This batch contains 2.68% total saponins, 0.033% astragaloside I and 0.10% ginsenoside Rb1 (see also Table 2).

The bacterial reverse mutation test (Zin, 2016, unpublished study report) was performed in compliance with good laboratory practice (GLP) and following OECD Test Guideline (TG) 471. The test was carried out with *Salmonella* Typhimurium strains TA98, TA100, TA1535, TA1537 and *Escherichia coli* strain WP2 *uvrA*, using the pre-incubation method in the presence and in the absence of an exogenous metabolic activation system (i.e. S9-mix). In order to evaluate the cytotoxicity of the test item a dose-finding test was performed with the five strains mentioned above, with and without metabolic activation, using the following five concentrations of the NF dissolved in water: 313, 625, 1,250, 2,500 and 5,000 μg/plate. There was no sign of cytotoxicity and no precipitation of the test material was observed at any concentration. Thus, the main test was performed using the same concentrations. Positive and negative controls were included. The NF induced no biologically relevant increase in the number of revertant colonies compared with the negative controls for all strains, in the presence and absence of S9-mix, in the dose finding test and the main test.

The *in vitro* mammalian cell gene mutation test using the hypoxanthine-guanine phosphoribosyl transferase (*Hprt*) gene (Ruslan et al., 2016, unpublished study report) was performed in compliance with GLP. According to the applicant, the test was conducted in accordance with OECD TG 476. However, the Panel notes that the NF was only tested in the absence and not in the presence of an exogenous metabolic activation system (S9-mix) in the main experiment, which is considered a relevant deviation. No rationale could be provided by the applicant for this deviation. In the absence of activation, the NF did not induce an increase in the number of gene mutations in mammalian cells.

Following an EFSA request, and in accordance with the EFSA recommendations for genotoxicity testing (EFSA Scientific Committee, 2011), the applicant submitted two *in vitro* mammalian cell micronucleus tests following OECD TG 487. As per the EFSA request, one micronucleus test per plant extract was carried out.

The micronucleus test (Vedic Lifesciences, 2019a, Study Nr. 190503/NL/PC, unpublished study report), submitted for the *P. notoginseng* extract, was performed with a batch (i.e. #20170702) that contained 1.08% total saponins and 0.31% ginsenoside Rb1. The test, which followed OECD TG 487, was carried out in cultured mammalian CHO-K1 cells. In order to evaluate the cytotoxicity of the test item, the cells were exposed to the extract (dissolved in water and diluted with culture medium) at concentrations of 310 μg/mL, 620 μg/mL, 1,250 μg/ml, 2,500 μg/mL and 5,000 μg/mL either for 30 h in the absence of an exogenous metabolic system (S9-mix) or for 3 h in the presence or absence of S9-mix. As the cytotoxicity even at the highest concentration of 5,000 μg/mL was less than 40%, the study coordinators decided to use the following four concentrations of the extract in the main experiment: 620, 1,250, 2,500 and 5,000 μg/mL. In the main test, the cytotoxicity at the highest concentration of 5,000 μg/mL for 30 h without S9-mix was 35.9%. After short-term exposure to this concentration in the presence of metabolic activation, the cytotoxicity was 38.5%, while without metabolic activation it was 27.8%. Appropriate positive controls were used as per OECD TG 487 and an adequate response was seen. Micronuclei were manually scored for at least 2,000 cells per concentration. Compared to the vehicle control, there were no statistically significant differences in the number of micronuclei at any tested concentration with and without metabolic activation.

Another micronucleus test was submitted (Vedic Lifesciences, 2019b; Study Nr. 190502/NL/PC, unpublished study report), which assessed the clastogenic/aneugenic potential of the *A. membranaceus* extract. The tested batch (i.e. #20180225) contained 3.1% total saponins and 0.12% astragaloside I. Also this test, which followed OECD TG 487, was carried out in cultured mammalian CHO-K1 cells. In order to evaluate the cytotoxicity of the test item, the cells were exposed to the extract (dissolved in water and diluted with culture medium) at concentrations of 310 μg/mL, 620 μg/mL, 1,250 μg/mL, 2,500 μg/mL and 5,000 μg/mL for 30 h in the absence or for 3 h in the presence and absence of an exogenous metabolic system (S9-mix). The observed toxicity was up to 29% after exposure for 30 h without S9-mix. After exposure for 3 h with and without metabolic activation, the cytotoxicity amounted to 24% and 18.1%, respectively. As the observed toxicity was less than 35% at any concentration with or without S9-mix, the study coordinators decided to use the following three concentrations of the extract in the main experiment: 500, 1,500 and 5,000 μg/mL. In the main test, the cytotoxicity at the highest concentration of 5,000 μg/mL for 30 h without S9-mix was 28.5%. After short-term exposure to this concentration in the presence of metabolic activation the cytotoxicity was 30.5%, while without metabolic activation it was 29.4%. Appropriate positive controls were used as per OECD TG 487 and an adequate response was seen. Micronuclei were manually scored for at least 2,000 cells per concentration. Compared to the vehicle control there were no statistically significant differences in the number of micronuclei at any tested concentration with and without metabolic activation.

The Panel concludes that the studies provided do not raise concerns for genotoxicity of the NF.

3.10.2. Subacute and subchronic toxicity

The applicant provided a 28-day oral toxicity study (Pasics Szakonyiné, 2011, unpublished study report) conducted in compliance with GLP and in accordance with OECD TG 407. Twenty Wistar rats (n = 10 per sex) per group were administered by gavage 0, 100, 300 or 1,000 mg/kg body weight (bw) per day of the NF for 28 days. The batch (#20100621) that was used in this study contained 2.35% total saponins, 0.17% ginsenoside Rb1 and 0.054% astragaloside I (see also Table 2). The test item was prepared daily by suspending the NF in 1%-aqueous methylcellulose vehicle.

There was no mortality during the study period. No clinical signs or abnormalities in behaviour, motor activity or general health were observed in control or treated animals. No relevant differences in body weight or body weight gain or feed consumption were observed among the groups.

A number of statistically significant differences in haematology and clinical chemistry between controls and animals administered the NF were observed (available in a file annexed to the scientific opinion under 'Supporting information'). The number of neutrophils significantly increased in all groups receiving the NF. In male rats, the number of basophils was reduced in the mid-dose group. In females, white blood cells (WBC) and lymphocytes were reduced in the low- and mid-dose groups and in the mid- and high-dose groups, respectively. Furthermore, in female rats, mean corpuscular volume

(MCV) was reduced in the low-dose group, mean corpuscular haemoglobin concentration (MCHC) was decreased in the mid-dose group and platelets were increased in the low-dose group. Activated partial thromboplastin time (APTT) was increased in the high-dose group compared to the control group. The serum concentration of phosphorus increased with dose in both sexes, being statistically significant in the high-dose group of males and in the mid- and high-dose groups for females.

Absolute and relative organ weights were not different between the groups, except for a higher testes to brain weight ratio in the low-dose males and a lower absolute and relative to body weight kidney weight in the high-dose males. However, the differences were small and considered biologically not relevant. No relevant dose-related histopathological findings were observed.

The applicant provided a repeated dose 90-day oral toxicity study (Upadhyaya and Wang, 2017, unpublished study report) which was conducted in accordance with OECD TG 408 and in compliance with GLP.

Wistar rats (n = 10/group and sex) of 6–8 weeks of age were randomly allocated to one of six groups, which were administered the NF daily by gavage for 90 consecutive days at dosages of 0 mg/kg bw per day (control group), 100 mg/kg bw per day, 300 mg/kg bw per day or 1,000 mg/kg bw per day. The study included two additional groups (i.e. recovery groups) that received 0 mg/kg bw per day (controls) or 1,000 mg/kg bw per day, respectively, for 90 days, and thereafter 0 mg/kg bw per day for an additional 2-week period (i.e. recovery period). The batch (#C20160606) that was used in this study contained 3.79% total saponins, 0.17% ginsenoside Rb1 and 0.028% astragaloside I (see also Table 1). The test item was prepared (with water as vehicle) every day before administration.

None of the animals died in the course of the study. No treatment-related clinical signs were observed in any of the animals throughout the study. Ophthalmological and neurobehavioural examinations of the animals did not reveal any differences between the groups. No treatment-related changes in body weight or body weight gain were observed. However, compared to the controls, feed consumption was lower in males in the low-dose group until day 78 and it was higher in females in the mid- and high-dose groups for most of the study duration. On day 91 (day 105 for the recovery groups), animals were subjected to necropsy and a gross pathological examination was performed. No changes were found in gross pathology.

A number of statistically significant differences in clinical chemistry between controls and animals exposed to the NF were observed (A summary of the results can be found in the online version of this output under the 'Supporting information' section: https://doi.org/10.2903/j.efsa.2020.6099). Serum cholesterol and triglyceride concentrations were increased in males and females. Cholesterol was increased in the high-dose group in the males and in females in all the groups that were administered the NF. Triglycerides were increased in the mid- and high-dose groups for both sexes. Increases were also observed for alanine amino transferase (ALT) (in males of the high-dose group and in females in all the treatment groups) and alkaline phosphatase (ALP) (in males of the high-dose group and in females in the low-dose group). Aspartate amino transferase (AST) was increased in females only, i.e. in the low- and mid-dose groups. Furthermore, lactate dehydrogenase (LDH) was increased in both sexes, in males in all treatment groups and in females in the high-dose group. In males, phosphorous was increased in the mid- and high-dose groups and remained higher (statistically significant) also after the recovery period. In females, succinate dehydrogenase (SDH) and total protein were increased (SDH in the mid- and high dose groups, total protein in all groups receiving the NF).

A number of statistically significant findings were observed for absolute and relative organ weights, but the observed changes were small, often not dose-related and in many cases a result of rather low values in the control groups (with respect to historical controls). No differences were seen in urinalysis.

The Panel notes that a considerable number of changes were observed in clinical chemistry, including markers of liver and kidney toxicity. Even though the size of individual changes was rather small and within historical ranges, the effects were seen consistently in both sexes, were often dose-related and, in the case of phosphorus, also observed after the recovery period. The Panel considers that taken together, the findings imply a potentially adverse effect of the NF. The Panel also considers that taking into account the magnitude of the effects seen, the overall lowest observed adverse effect level (LOAEL) is to be set at 300 mg/kg bw per day. Consequently, the Panel considers the lowest dose tested in the study, i.e. 100 mg/kg bw per day, as the overall no observed adverse effect level (NOAEL).

3.10.3. Human data

The applicant did not provide any human studies performed with the NF nor were there any human studies with the NF retrieved by extensive literature searches.

3.11. Allergenicity

The Panel notes the protein content (2–5%) of the NF and, thus, the potential of the NF to elicit allergic reactions.

The literature searches carried out by the applicant and by the EFSA contractor (UCT Prague) did not retrieve any studies reporting incidents of allergenicity to the NF or to *A. membranaceus*, one of the two plants that is used to produce the NF.

With respect to *P. notoginseng*, the applicant submitted one review (Dharmananda, 2004), which described 19 case reports of allergic reactions to Sanqi (i.e. dried tuber of *P. notoginseng*). No further information on the allergenicity of *P. notoginseng* was provided.

The Panel notes the limited epidemiological data reported on one of the two plants that is used to produce the NF.

Overall, taking into account the long history of use of both plants (i.e. *A. membranaceus* and *P. notoginseng*) that are used to produce the NF, the Panel considers that the risk of allergic reactions to the NF for the general population is unknown but expected to be low.

4. Discussion

The NF is a mixture of the root extracts of *A. membranaceus* and *P. notoginseng*, and contains marker compounds within a specified range (i.e. 1.5–5% total saponins, 0.1–0.5% ginsenoside Rb1 and 0.01–0.1% astragaloside I). The Panel considers that the information provided on the composition of the NF and the production process is sufficient and does not raise safety concerns.

The applicant proposed to use the NF as a food supplement for the general adult population (with the exception of pregnant women) at a maximum daily amount of 350 mg. The Panel considers that, taking into account the proposed conditions of use, the consumption of the NF is not nutritionally disadvantageous.

The provided genotoxicity studies do not raise concerns for genotoxicity of the NF. In view of the maximum daily amount proposed for the NF, contribution of pesticides or contaminants is not of toxicological relevance.

Based on the findings of a subchronic toxicity study, supported by a subacute toxicity study, the Panel identified the overall NOAEL of the NF at 100 mg/kg bw per day.

The Panel considers an uncertainty factor (UF) of 200 appropriate, in order to account for inter- and intra-species variability (UF 10 $\times$ 10) and for the lack of chronic toxicity data (additional UF of 2). The application of this UF to the NOAEL results in a safe intake level for the NF of 0.5 mg/kg bw per day. Considering a standard bodyweight of 70 kg for adults (EFSA Scientific Committee, 2012), this level corresponds to a maximum daily intake of 35 mg NF for the target population, i.e. adults excluding pregnant women.

5. Conclusions

The Panel concludes that the NF, i.e. a botanical extract derived from both *P. notoginseng* and *A. membranaceus* roots (AstraGin™), is safe at an intake level of 0.5 mg/kg bw per day. This level corresponds to a maximum intake of 35 mg/day for the target population, i.e. adults excluding pregnant women.

The Panel could not have reached the conclusion on the safety of the NF under the proposed conditions of use without the toxicological data for which protection of proprietary data was requested by the applicant (Pasics Szakonyiné, 2011; Zin, 2016; Upadhyaya and Wang, 2017; Vedic Lifesciences, 2019a,b).

Steps taken by EFSA

1) Letter from the European Commission to the European Food Safety Authority with the request for a scientific opinion on the safety of a botanical extract derived from *Panax notoginseng* and *Astragalus membranaceus*. Ref. Ares(2018)5414664, dated 22 October 2018.

2) On 22 October 2018, EFSA received a valid application from the European Commission on a botanical extract derived from *Panax notoginseng* and *Astragalus membranaceus* (AstraGin™) as a novel food, which was submitted by NuLiv Science (US), and the scientific evaluation procedure started.
3) On 6 March 2019, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4) On 30 August 2019, additional information was provided by the applicant and the scientific evaluation was restarted.
5) On 11 November 2019, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
6) On 23 January 2020, additional information was provided by the applicant and the scientific evaluation was restarted.
7) During its meeting on 24 March 2020, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of a botanical extract derived from *Panax notoginseng* and *Astragalus membranaceus* as a NF pursuant to Regulation (EU) 2015/2283.

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Abbreviations

ADME	absorption, distribution, metabolism and excretion
ALP	alkaline phosphatase
ALT	alanine amino transferase
APTT	activated partial thromboplastin time
AST	aspartate amino transferase
bw	body weight
CFU	colony forming units
ELSD	evaporative light scattering detector
FDA-BAM	Food and Drug Administration's Bacteriological Analytical Manual
GC-MS	gas chromatography–mass spectrometry
GLP	good laboratory practice
GMP	good manufacturing practice
HACCP	hazard analysis and critical control points
HPLC	high-performance liquid chromatography
HPRT	hypoxanthine-guanine phosphoribosyl transferase gene
ICP-MS	inductively coupled plasma mass spectrometry
LDH	lactate dehydrogenase
LOAEL	lowest observed adverse effect level
LOD	limit of detection
LOQ	limit of quantification
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
MF	mutation frequency
NDA	EFSA Panel on Dietetic Products, Nutrition and Allergies

NF	novel food
NOAEL	no observed adverse effect level
ODAP	oxalyldiaminopropionic acid
OECD	Organisation for Co-operation and Development
RNA	ribonucleic acid
SDH	succinate dehydrogenase
SLE	systemic lupus erythematosus
TG	test guidelines
UF	uncertainty factor
UV	ultraviolet
WBC	white blood cells