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Safety of EstroG-100™ as a novel food pursuant to Regulation (EC) No 258/97 (Scientific Opinion)

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)

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Safety of EstroG-100™ as a novel food pursuant to Regulation (EC) No 258/97

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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 EstroG-100™ as a novel food (NF) submitted pursuant to Regulation (EC) No 258/97 of the European Parliament and of the Council. The NF is EstroG-100™, a hot-water extract of a mixture of three herbal roots (*Cynanchum wilfordii* Hemsley, *Phlomis umbrosa* Turcz. and *Angelica gigas* Nakai*),* which is concentrated and spray-dried. The information provided on the composition, specifications and stability of the NF is sufficient, and does not raise safety concerns. The applicant intends to use EstroG-100™ in food supplements, with a proposed maximum intake level of 514 mg/day. The target population is post-menopausal women. The Panel considers that the information provided does not raise safety concerns as regards the genotoxicity of the NF. The Panel considers that the no-observed-adverse effect level (NOAEL) derived from the subchronic 90-day oral toxicity study with EstroG-100™, which was supported by observations in other studies, is 500 mg/kg body weight (bw) per day. Taking into account the NOAEL and the proposed maximum intake level, the Panel considers that the margin of safety of 68 is not sufficient. Based on the absence of chronic toxicity data, increase in effects with exposure duration in toxicity studies, and the absence of investigations of liver parameters and haematology in human studies, the Panel applies the uncertainty factor of 200 to derive the maximum safe intake level for the NF. Thus, the Panel concludes that the NF, EstroG-100™, is safe for the use in food supplements at the maximum intake level of 175 mg/day for an adult of 70 kg bw.

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Keywords: EstroG-100™, *Cynanchum wilfordii*, *Phlomis umbrosa*, *Angelica gigas*, novel food, safety

Requestor: European Commission following an application by Naturalendo Tech Co., Ltd

Question number: EFSA-Q-2015-00249

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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 EstroG-100™ as a novel food (NF) submitted pursuant to Regulation (EC) No 258/97 of the European Parliament and of the Council. The assessment follows the methodology set 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. The assessment is based on the 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.

The NF is EstroG-100™, an extract of a mixture of three herbal roots: *Cynanchum wilfordii* Hemsley (32.5%), *Phlomis umbrosa* Turcz. (32.5%) and *Angelica gigas* Nakai (35%). The mixture of the three herbal roots is hot-water extracted, concentrated and spray-dried.

The information provided on the composition, specifications and stability of the NF is sufficient and does not raise safety concerns. The production process is sufficiently described and does not raise concerns about the safety of the NF.

The applicant intends to use EstroG-100™ in food supplements (as tablets, powder, capsules, softgel or gelcaps). The proposed maximum intake level is 514 mg/day of EstroG-100™. The target population is post-menopausal women.

Taking into account the composition of the NF and the intended use levels, the Panel considers that the NF does not have a nutritionally relevant role in the human diet and that the consumption of the NF is not nutritionally disadvantageous.

The Panel considers that the information from a bacterial reverse mutation test, *in vitro* chromosome aberration test and *in vivo* micronucleus test does not raise safety concerns as regards the genotoxicity of the NF.

In a subchronic 90-day repeated dose oral toxicity study, EstroG-100™ was administered to Sprague–Dawley rats, via oral intubation, at doses of 0, 500, 1,000, or 2,000 mg/kg body weight (bw) per day. This study reported dose-related effects at the dose of 1,000 mg/kg bw per day (e.g. increase in albumin, total protein levels, reticulocytes, absolute and relative liver weights). Some of these effects were also observed in a repeated dose 28-day oral toxicity study with EstroG-100™ and repeated dose 90-day oral toxicity studies with the single plants which constitute EstroG-100™. The Panel considers that the no-observed-adverse effect level (NOAEL) derived from the subchronic 90-day oral toxicity study with EstroG-100™, which was supported by observations in the other studies, is 500 mg/kg bw per day.

Two double-blind, placebo-controlled studies in women, who consumed EstroG-100™ or Estromon®, which contained minerals and vitamins in addition to EstroG-100™, were provided by the applicant. The Panel considers that these studies do not raise safety concerns with respect to the limited clinical chemical parameters investigated in relation to EstroG-100™. However, haematological and liver effects, which appeared in the toxicological 90-day repeated dose study, have not been investigated in these studies.

Based on the observations from animal and *in vitro* studies on potential oestrogenic effect of the NF, the Panel considers that EstroG-100™ does not exhibit classical oestrogenic effects.

Taking into account the NOAEL of 500 mg/kg bw per day and the proposed maximum intake level of 514 mg/day for the NF, which corresponds to 7.3 mg/kg bw per day for an adult of 70 kg bw, the Panel considers that the margin of safety of 68 (i.e. ratio between the NOAEL and the proposed maximum intake level), is not sufficient. Based on the absence of chronic toxicity data, an increase in effects with exposure duration in toxicity studies (effects on liver (weight, albumin, total protein) in the 90-day repeated dose study as compared to the 28-day repeated dose study), and the absence of investigations of liver parameters and haematology in human studies, the Panel applies the uncertainty factor of 200 to derive the maximum safe intake level for the NF. Thus, by applying the uncertainty factor of 200 to the NOAEL, the Panel derives the maximum intake level of 2.5 mg/kg bw per day for the NF, which corresponds to 175 mg/day for an adult of 70 kg bw.

The Panel concludes that the NF, EstroG-100™, is safe for the use in food supplements at the maximum intake level of 175 mg/day for an adult of 70 kg bw.

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1. Introduction

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

On 13 March 2014, the company Naturalendo Tech Co., Ltd submitted a request under Article 4 of the Novel Food Regulation (EC) No 258/97[1] to place on the market EstroG-100™ as a novel food (NF).

On 29 July 2014, the competent authority of Ireland forwarded to the Commission its initial assessment report, which came to the conclusion that EstroG-100™ meets the criteria for acceptance of a NF defined in Article (3)1 of Regulation (EC) No 258/97.

On 15 September 2014, the Commission forwarded the initial assessment report to the other Member States (MS). Several of the MS submitted comments or raised objections.

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

- The recognised botanical name for '*Phlomis umbrosa* Turcz.' is '*Phlomoides umbrosa* (Turcz.) Kamelin & Makhm' (The Plant List, 2013).
- Information is missing on the origin of the plants used as raw materials and how they were obtained, on the relevant ingredients of the plants used, on standardisation of the extract used, and whether hazard-analysis and critical control points (HACCP) concept was applied.
- The specifications of the NF, which is limited to three 'markers', is considered insufficient to characterise the NF.
- No information has been provided on the presence of other plant-derived compounds (such as saponins, flavonoids).
- A MS expressed concerns on the presence of potentially toxic compounds in the NF.
- Specifications for the water used in extraction should be provided.
- The 'extraction ratio' for each individual root in the NF is not clearly presented. Furthermore, different analytical methods are used for the selected markers. The lowest level specified for each marker in the NF does not seem to be consistent with the 'plant-to-extract ratio'.
- No information was provided whether the laboratories where the analyses were conducted have been accredited under an internationally recognised scheme for the various analyses.
- The stability of the preparation has been set at 3 years, although the moisture of the batches used in the stability study is unknown.
- A flow chart on the production process for EstroG-100™ is missing.
- Clarification is needed on the use of methanol as an extraction solvent for the raw materials.
- Although the application refers to the possibility of using freeze-drying, the conditions for this process have not been described.
- Information on post-marketing surveillance of EstroG-100™ in the countries where it has been sold (e.g. USA and Canada) is needed.
- Information should be provided on the consumption of the three plants as raw materials for foodstuffs in Asia.
- Human data on pharmacokinetics of decursin/decursinol angelate, which are compounds in *Angelica gigas* Nakai, are required.
- Genotoxicity studies on components, which constitute the NF, should be provided.
- As regards to the subchronic oral toxicity study by Moon et al. (2009), some MS expressed concerns that the test material used in this study was not equivalent to the NF. The calculated safety margin between the apparent no-observed-adverse effect level (NOAEL) and the proposed daily dose of the NF is considerably lower compared to the uncertainty factors advised by the European Food Safety Authority (EFSA) (http://www.efsa.europa.eu/en/efsajournal/pub/2579.htm). Therefore, the available toxicity data is insufficient to support a safe use of the NF as proposed by the applicant.
- The human safety data are restricted to the 12-week study by Chang et al. (2012) as the study by Lee et al. (2005) was carried out with a different product. The study by Chang et al. (2012) is considered to be inadequate to identify possible side effects, owing to small size groups and the short study duration.

[1] Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. OJ L 43, 14.2.1997, p. 1–6.

- A clinical study with EstroG-100™ in European post-menopausal women, for at least 1 year of duration, is requested. If the NF is intended to be used by pre- or peri-menopausal women, reproductive/developmental data are needed.
- There is insufficient information on possible oestrogenic effect of the NF. Possible hormonal effects of the NF should be examined in appropriate studies, which are recommended in the OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupters (e.g. studies to measure oestrogenic activity by means of transactivation assays, proliferation tests on breast cancer cells, tests for uterotrophic effects).
- No information has been provided on the mode of action of the NF.
- An assessment of the three plants should be undertaken to show the botanical relatedness to existing food allergens. The potential to elicit reaction in the allergic population also should be considered. The roots are likely to contain homologues of the major birch pollen allergen Bet v 1 which due to their level of structure and sequence, conservation across plant species are highly likely to elicit an allergic reaction in sensitised individuals. It is highly likely that the root of *A. gigas* Nakai, which is related to *Apium graveolens*, contains allergens which are likely to pose a risk to individuals with celery allergy.
- Allergenicity deserves attention and monitoring of allergic effects when the NF is released.

In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002[2], EFSA is asked to carry out the additional assessment for EstroG-100™ as a NF in the context of Regulation (EC) No 258/97.

EFSA is asked to carry out the additional assessment and to consider the elements of a scientific nature in the comments raised by the other MS.

2. Data and methodologies

2.1. Data

The assessment of the safety of this 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 MS and the responses of the applicant.

In accordance with Commission Recommendation 97/618/EC[3], EstroG-100™ is allocated to Class 2.2, i.e. foods or food ingredients that are 'complex novel food from non-genetically modified source; 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 2.2, 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 3.1–3.9. The intention is to use the NF in food supplements (as tablets, powder, capsules, softgel or gelcaps). This assessment concerns only risk that might be associated with consumption of the NF at the proposed conditions of use, and is not an assessment of the efficacy of EstroG-100™ 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.

[2] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24.

[3] 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.

3. Assessment

3.1. Specification of the Novel Food (NF)

The NF is EstroG-100™, an extract of a mixture of three herbal roots: *Cynanchum wilfordii* Hemsley (32.5%), *Phlomis umbrosa* Turcz. (32.5%) and *Angelica gigas* Nakai (35%). The mixture of the three herbal roots is hot-water extracted and concentrated, and dried by spray drying.

Cynanchum wilfordii Hemsley (from the former family Asclepiadaceae) is the scientific name of this plant. Synonyms are: *Cynoctonum wilfordii* Maxim., *Seutera wilfordii* (Maxim.) Pobed., *Vincetoxicum wilfordii* (Maxim.) Franch. & Sav. The plant is also called *Cybanchi wilfordi* Radix; Ge Shan Niu Pi Xiao or Baishouwu in China; Baek-ha-su-o or Baek-su-o or Eun-jo-rong in Korea. The used part of the plant is the root. This plant contains various phytochemicals such as α-methylglaucobioside, β-methylglaucobioside, β-sitosterol, caudatin, cinnamic acid, cymaronic acid, D-cymarose, deacetyl-metaplexigenin, glaucogenin A, kidjoranine, L-cymarose, methyleugenol, lineolone, 12-*O*-cinnamoyl-20-*O*-ikemaoyl-sarcostin, 12-*O*-cinnamoyl-20-*O*-tigloylsarcostin, penupogenin, sarcostin, wilforibiose and wilfoside (Jiang et al., 2011; TradiMed database).

The botanical name of *Phlomis umbrosa* Turcz. (from the Lamiaceae family) is *Phlomoides umbrosa* (Turcz.) Kamelin & Makhm. The plant is also called Phlomidis Radix.; Cao su or Xu duan in China and Sok-dan in Korea. The used part of the plant is the root. *P. umbrosa* contains various phytochemicals such as betonicine, shanzhiside methyl ester and succinic acid. Several studies were performed on *P. umbrosa* in order to determine the composition of the root (Guo et al., 2001; Liu et al., 2007, 2008, 2009a,b; Deng et al., 2011).

Angelica gigas Nakai (from the Umbelliferae family) is the scientific name of this plant. The plant is also called *Angelicae gigantis* or Dong Quai in China and Dang-gui in Korea. The used part of the plant is the root. Decursin, decursinol angelate, nodakenin and decursinol have been identified in *A. gigas* (Konoshima et al., 1968; Kim et al., 2006; Cho et al., 2007; Avula et al., 2007; Ahn et al., 2008; Kim et al., 2011).

Information on the origin of the three plants that are used as raw materials (e.g. place of cultivation, harvesting, and processing) was provided by the applicant. *C. wilfordii* Hemsley and *A. gigas* Nakai are cultivated and harvested in the central part (Chungcheong province) and eastern part (Gangwon province) of South Korea and collected by the regional Agricultural Association. *P. umbrosa* Turcz. is collected in the central part of South Korea (Chungcheong province) and in China (Sichuan and Hubei provinces). After being processed (washing, drying, cutting and packaging), the plants are transported to the manufacturing facilities.

The three main families of compounds present in EstroG-100™ are coumarins, iridoids and phenols.

Each individual root was tested for its identity. The roots were extracted with methanol and analysed by chromatography against markers, which were representative of each plant: shanzhiside methyl ester was used as marker of the iridoids class for *P. umbrosa* Turcz. and nodakenin was used as a marker of the coumarins class for *A. gigas* Nakai. For *C. wilfordii* Hemsley, owing to the absence of a reference for wilfosides, the applicant proposed to analyse *C. wilfordii* Hemsley against cinnamic acid, since its content is proportional to that of wilfoside, which belongs to the class of saponins (Tsukamoto et al., 1985). For *C. wilfordii* Hemsley, an alkaline hydrolysis step is added before extraction with methanol in order to analyse free cinnamic acid liberated from wilfoside by high-performance liquid chromatography (HPLC). Each individual root was checked for compliance to specifications in terms of appearance, each individual marker, loss on drying and heavy metals.

Upon EFSA's request for clarifications on the identity of the three roots, the applicant indicated that cinnamic acid, which has been proposed as a specific marker for *C. wilfordii* Hemsley, was not detected in *P. umbrosa* Turcz. or *A. gigas* Nakai. However, the applicant acknowledged that cinnamic acid can be found in other *Cynanchum* plants (e.g. *Cynanchum auriculatum* Royle ex Wight). In this respect, the applicant indicated that the Ministry of Food and Drug Safety of Korea requires analyses by polymerase chain reaction (PCR) for controlling the absence of *C. auriculatum* in products derived from *C. wilfordii.* The applicant assured that quality controls by PCR are performed on each batch of the root and that, therefore, the possibility of misuse of *C. auriculatum* Royle ex Wight is eliminated.

Following an EFSA request for clarification on the composition and specifications of EstroG-100™, the applicant provided the results on the analyses of several batches of EstroG-100™ for the classes of compounds which are naturally occurring in the three plants, together with the respective methods of detection. The applicant indicated that *A. gigas* Nakai has been suspected to be a source of methoxsalen (or xanthotoxin or 8-methoxypsoralen – CAS number 298817), which is a furanocoumarin

found naturally in several plants (e.g. *Ammi majus*, *Fagara xanthoxyloïdes*, *Citrus bergamia*). Methoxsalen is also found in *Angelica archangelica* (a different species than the one used in EstroG-100™). Methoxsalen is known to induce phototoxic effects. Since the applicant could not exclude the presence of methoxsalen in the NF, a maximum level has been included in the specification.

The specifications for the NF and the respective methods of analysis as proposed by the applicant are presented in Table 1. Test results for five batches show compliance with the specifications (Table 2). The applicant indicated that accredited and validated methods of the Korean Food Code or from published papers were used in the analyses applied for the specifications.

Table 1: Proposed specifications of the NF

Parameter	Specification	Method
Appearance	Yellowish brown fine powder	Visual
Loss on drying	NMT 100 mg/g	KFC <9.1.1.1.1>
Assay		
Cinnamic acid[(a)]	0.012–0.039 mg/g	HPLC[(e)]
Shanzhiside methyl ester[(b)]	0.20–1.55 mg/g	HPLC
Nodakenin[(c)]	3.35–10.61 mg/g	HPLC
Methoxsalen[(d)]	NMT 3 mg/g	HPLC
Phenols	13.0–40.0 mg/g	UV-visible spectrophotometry
Coumarins	13.0–40.0 mg/g	UV-visible spectrophotometry
Iridoids	13.0–39.0 mg/g	UV-visible spectrophotometry
Saponins	5.0–15.5 mg/g	UV-visible spectrophotometry
Nutritive components		
Carbohydrates	600–880 mg/g	<KFC 9.1.1.4/General Test_Carbohydrate>
Proteins	70–170 mg/g	<KFC 9.1.3.1/General Test_Total nitrate and Crude Protein>
Fats	NMT 4 mg/g	<KFC 9.1.1.5.1/General Test_Crude Fat>
Microbiological parameters		
Total viable plate count	NMT 5,000 CFU/g	KFC <9.3.5.1>
Total mould and yeast	NMT 100 CFU/g	KFC <9.3.10>
Coliform bacteria	NMT 10 CFU/g	KFC <9.3.7.2>
Salmonella	Negative/25 g	KFC <9.3.11>
Escherichia coli	Negative/25 g	KFC <9.3.8>
Staphylococcus aureus	Negative/25 g	KFC <9.3.12>
Heavy metals		
Lead	NMT 0.65 mg/kg	KFC <9.7.1.2.1>
Arsenic	NMT 3.0 mg/kg	KFC <9.7.1.2.3>
Mercury	NMT 0.1 mg/kg	KFC <9.7.1.2.4>
Cadmium	NMT 1.0 mg/kg	KFC <9.7.1.2.2>

CFU: colony forming units; HPLC: high-performance liquid chromatography; KFC: Korean Food Code; LOQ: limit of quantification; NMT: not more than.
(a): LOQ for cinnamic acid: 2.2×10^{-5} mg/g.
(b): LOQ for shanzhiside methyl ester: 6.1×10^{-4} mg/g.
(c): LOQ for nodakenin: 4.1×10^{-4} mg/g.
(d): LOQ for methoxsalen: 8.7×10^{-4} mg/g.
(e): Alkaline hydrolysis is performed before the HPLC assay is applied.

Table 2: Analytical results for testing of five batches of the NF

Parameter	Specification	SE14010	SE14011	SE14012	SE14014	EG-SD01-140819
Appearance	Yellowish brown fine powder	Complies	Complies	Complies	Complies	Complies
Loss on drying (mg/g)	NMT 100	36	31.8	39	35	46.7
Assay						
Cinnamic acid (mg/g)	0.012–0.039	0.015	0.015	0.018	0.025	0.021
Shanzhiside methyl ester (mg/g)	0.20–1.55	0.22	0.22	0.22	0.31	0.22
Nodakenin (mg/g)	3.35–10.61	5.33	5.33	5.46	4.28	5.29
Methoxsalen (mg/g)	NMT 3	0.130	0.127	0.132	0.124	0.107
Phenols (mg/g)	13.0–40.0	28.0	33.6	28.8	30.3	26.8
Coumarins (mg/g)	13.0–40.0	29.9	33.6	34.4	30.0	35.2
Iridoids (mg/g)	13.0–39.0	25.3	28.5	28.5	38.3	28.3
Saponins (mg/g)	5.0–15.5	8.8	12.0	9.9	9.3	12.0
Nutritive components						
Carbohydrates (mg/g)	600–880	814	719	809	789	727
Proteins (mg/g)	70–170	91	136	93	108	136
Fats (mg/g)	NMT 4	1	1	1	2	1
Microbiological parameters						
Total viable count CFU/g	NMT 5,000	200	2,000	750	0	400
Total mould & yeast CFU/g	NMT 100	0	0	0	0	0
Coliform bacteria CFU/g	NMT 10	Not detected	Not detected	Not detected	Not detected	Not detected
Salmonella	Negative/25 g	Negative	Negative	Negative	Negative	Negative
Escherichia coli	Negative/25 g	Negative	Negative	Negative	Negative	Negative
Staphylococcus aureus	Negative/25 g	Negative	Negative	Negative	Negative	Negative
Heavy metals						
Lead (mg/kg)	NMT 0.65	0.06	0.14	0.13	0.13	0.10
Arsenic (mg/kg)	NMT 3.0	0.33	0.26	0.31	0.50	0.35
Mercury (mg/kg)	NMT 0.1	0.00	0.00	0.00	0.00	0.00
Cadmium (mg/kg)	NMT 1.0	0.01	0.01	0.02	0.02	0.01

CFU: colony forming units; NMT: not more than.

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 EstroG-100™ was tested in three batches in a polyethylene film in a fibre drum, up to 3 years, at room temperature. The three markers (cinnamic acid, shanzhiside methyl ester and nodakenin), coliform bacteria, appearance and loss on drying were the parameters tested. Results show that EstroG-100™ is stable for a period of 3 years at room temperature. Therefore, the shelf life of the NF was proposed to be 3 years.

On the basis of the data provided, the nature of product and the low water content, the Panel considers that the NF is sufficiently stable.

3.2. Effect of the production process applied to the NF

The roots of *C. wilfordii* Hemsley, *P. umbrosa* Turcz. and *A. gigas* Nakai, after inspection and testing for the standard compounds (cinnamic acid for *C. wilfordii* Hemsley, shanzhiside methyl ester for *P. umbrosa* Turcz. and nodakenin for *A. gigas* Nakai) and contaminants, are weighed in the established ratio (32.5:32.5:35) and extracted with hot water. Then, the water extract is filtered in order to

remove insoluble materials and concentrated and sterilised. Finally, the product is spray-dried. After the final inspections to check for compliance with specifications, EstroG-100™ is packed.

A flow chart of the manufacturing process has been provided by the applicant.

Noting that the extraction process occurs with hot water, the Panel considers that lipophilic compounds (such as methyleugenol) would not be expected to be present in the final product.

In response to comments from a MS, the applicant indicated that purified water, which is obtained by a hyperfiltration process through reverse osmosis, is the sole solvent used for the extraction process and no other solvents are used in the manufacturing process for the NF.

In response to comments from a MS, the applicant indicated that the manufacturing facility for EstroG-100™ follows the Good Manufacturing Practice (GMP) as requested by the Korean Health Functional Food Act and has been certified by the Korean Food and Drug Administration (KFDA). The applicant provided the summary of the HACCP procedures which are applied in the manufacturing facility.

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

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

EstroG-100™ is a hot-water extract of a mixture of herbal roots of *C. wilfordii* Hemsley, *P. umbrosa* Turcz. and *A. gigas* Nakai.

These herbal roots have been described in the medical book Dong-Eui-Bo-Gam, a traditional book in eastern Asia area, since 1600. These herbal roots are cited in the Korean Food Code 2006.

Cynanchum wilfordii has been used for centuries for traditional herbal food and is classified as a raw material for food by KFDA. The oldest record on *C. wilfordii* Hemsley (named as Baishouwu or He Shou Wu) is in Kaibao Bencao written in AD 1108, Song Dynasty in China. This plant has been used for more than 390 years in northern China and Korea. *C. wilfordii* has been used widely in Korea by oriental health professionals.

Phlomis umbrosa has been used for centuries for traditional herbal use and is classified as a raw material for food by KFDA. *P. umbrosa* has been used more than 390 years in Ningxia of northern China and Korea and it has been included in various traditional herbal remedies.

Angelica sinensis has been originally classified as plant for tea or other drinks by KFDA. The edible parts are leaf and root. There are three species in eastern Asia which are used as Dang-gui (Dong Qui): *A. sinensis* in China, *A gigas* in Korea, *Angelica acutiloba* Kitagawa in Japan. *A. gigas* has been used more than 390 years in Korea.

3.4. Anticipated intake/extent of use of the NF

The applicant intends to use EstroG-100™ in food supplements (as tablets, powder, capsules, softgel or gelcaps). The target population is post-menopausal women.

The proposed maximum intake level is 514 mg/day of EstroG-100™. The serving level of EstroG-100™ is 257 mg per capsule or tablet which are intended to be consumed twice a day with water. Since people in the European Union (EU) do not traditionally consume *C. wilfordii* Hemsley, *P. umbrosa* Turcz. or *A. gigas* Nakai either separately or mixed, the total intake of the extract of the three herbal roots corresponds to the consumption of EstroG-100™.

3.5. Information from previous exposure to the NF or its source

The three herbal roots have been used traditionally in Korea in a separate or mixed way.

EstroG-100™ has been on the market in South Korea since 2002. Sales volumes for 1 month packages of EstroG-100™ have been provided. From a post-marketing surveillance, which has been in place since 2006, a total of 291 complaints have been received for 10 million of 1 month packages of EstroG-100™ sold (i.e. 0.0029%). The applicant indicated that neither serious adverse events nor serious consumer complaints have been reported since 2002 in South Korea. In 2012, EstroG-100™ was recognised as a functional ingredient for health/functional food by KFDA.

EstroG-100™ was registered in 2010 and 2011 in the USA and Canada, respectively. The applicant provided sales volumes of EstroG-100™ in the USA and in Canada for 2014. No post-marketing surveillance is in place in the USA and Canada.

3.6. Nutritional information on the NF

The applicant provided information on the nutritional composition of 100 g of EstroG-100™: energy 309.8 kcal, 73.3 g of carbohydrate, 6.2 g of sugars, 12.6 g of crude protein, 0.4 g of crude fat, 169.9 mg of sodium and 18.7 g of dietary fibre.

Taking into account the composition of the NF and the intended use levels, the Panel considers that the NF does not have a nutritionally relevant role in the human diet and that the consumption of the NF is not nutritionally disadvantageous.

3.7. Microbiological information on the NF

EstroG-100™ is produced under GMP with good hygienic practices. Batches of the NF were screened for a range of microbiological contaminants and complied with the specifications (Table 2).

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

3.8. Toxicological information on the NF

The applicant provided unpublished reports on studies carried out with the NF: a bacterial reverse mutation test, an *in vitro* chromosome aberration test, an *in vivo* micronucleus test, a single-dose acute toxicity study, and repeated dose 28-day and 90-day oral toxicity studies.

In reply to a request for additional information from EFSA, the applicant performed a literature search to retrieve safety studies on the three plants that constitute EstroG-100™, as well as for constituents of these plants.

In reply to a comment from a MS, the applicant indicated that reproductive/developmental studies are not deemed to be necessary as EstroG-100™ is intended to be exclusively used by post-menopausal women.

3.8.1. Absorption, distribution, metabolism and excretion

In reply to a comment from a MS on the need of information on pharmacokinetics of decursin and its derivatives, the applicant provided three animal studies which reported that decursin and its derivatives are rapidly absorbed in rats (t_{max} = 1 h) and excreted at 90% within 36 h (Kim et al., 2009a; Song et al., 2011).

3.8.2. Genotoxicity

The applicant provided three unpublished reports on studies which investigated the potential mutagenicity of EstroG-100™ (Biotoxtech Co, 2010a–c). These studies were conducted in compliance with KFDA principles on good laboratory practice (GLP) and standards on toxicity studies (KFDA, 2009a,b).

In the bacterial reverse mutation test, four *Salmonella* Typhimurium strains (TA98, TA100, TA1535 and TA1537) and one *Escherichia coli* strain (WP2uvrA (pKM101)) were exposed to EstroG-100™ at levels up to 5,000 μg/plate, in the presence or absence of metabolic activation (S9), using the plate-incorporation and pre-incubation methods (Biotoxtech Co, 2010a). This test consisted of a 'dose-range finding' and the 'main study'. No growth inhibition and no precipitation of the test substance were evident at any dose levels, in the presence or absence of S9. In the 'main study', the mean number of revertant colonies was below the negative control at all doses both in the presence or absence of S9. This study did not show mutagenicity of EstroG-100™, at levels up to 5,000 μg/plate, in the presence or absence of S9.

In the *in vitro* chromosome aberration test, Chinese Hamster Lung (CHL/IU) cells were incubated with EstroG-100™ for 6 h, with or without metabolic activation (S9 mix) (short-time treatment) or 24 h without S9 mix (continuous treatment) (Biotoxtech Co, 2010b). This test consisted of the 'growth inhibition study' and the 'main study'. In the 'growth inhibition study', cytotoxicity was not evident following short-time and continuous treatments. Precipitation of the test substance was evident at 1,000, 2,500, and 5,000 μg/mL in short-time and continuous treatments. In the 'main study', the frequency of chromosomal aberrations in short-time and continuous treatments was less than 5% at all dose levels tested (1,250, 2,500 and 5,000 μg/mL). Precipitation of the test substance was evident at all dose levels in short-time and continuous treatments. This study did not show chromosome aberrations of EstroG-100™, at levels up to 5,000 μg/mL, with or without S9 mix.

In the *in vivo* micronucleus test (Biotoxtech Co, 2010c), 6–7-week-old mice (Crlj:Ori:CD1(ICR)) were administered EstroG-100™ by oral gavage. This test consisted of the 'dose-range finding study', the 'bone marrow collection study' and the 'main study'. In the 'dose-range finding study', which tested dose levels up to 2,000 mg/kg body weight (bw), no treatment related mortality or clinical signs were reported in any animals at any doses. In the 'bone marrow collection study', which tested the dose of 2,000 mg/kg bw, there was no toxicity at the bone marrow and the number of micronucleated polychromatic erythrocytes (MNPCE) in polychromatic erythrocytes (PCE) did not increase at 24, 48 and 72 h after dosing. In the 'main study' mice were given a single dose of 0, 500, 1,000, 2,000 mg/kg bw of EstroG-100™ and bone marrow cells were collected 24 h after dosing. No statistically significant increases or evidence of a dose-related effect were observed in the number of MNPCE in PCE at any dose tested as compared to the negative control group. There were no statistically significant differences in the number of PCE's in any dose as compared to the negative control group. No mortality or clinical signs were observed in any animal and no statistically significant differences in body weights were observed in any dose as compared to the negative control groups. This study did not show mutagenic effects of EstroG-100™, at levels up to 2,000 mg/kg bw. The Panel notes that it has not been demonstrated that the test material, which is a complex mixture of compounds, reached the bone marrow. However, the Panel acknowledges the high dose of the material which has been tested in this *in vivo* study.

Upon EFSA's request, the applicant provided one publication and four unpublished study reports on bacterial reverse mutation tests and *in vitro* chromosome aberration tests on a water extract of lyophilised *A. gigas* root, a micronised powder of *C. wilfordii* Hemsley and a micronised powder of *P. umbrosa* Turcz. (TTC Co, 2013a–d, Yun et al., 2015). Yun et al. (2015) also reported results from an *in vivo* micronucleus test where mice were orally given a water extract of lyophilised *A. gigas* root at doses up to 2,000 mg/kg bw per day for 4 days. These studies did not show genotoxicity or mutagenic effects of the plants investigated.

In reply to a comment from a MS and upon EFSA's request, the applicant provided publications of genotoxicity studies on compounds from *A gigas* Nakai (i.e. decursin and decursinol angelate) (Kim et al., 2009b; Mahat et al., 2012) and compounds from *C. wilfordii* Hemsley (i.e. esters and alcohols of cinnamic acid and cinnamyl alcohol) (Belsito et al., 2007). These studies did not show mutagenic effects of the compounds investigated.

The Panel considers that the information provided does not raise safety concerns as regards the genotoxicity of the NF.

3.8.3. Acute toxicity studies

The potential toxicity of the NF was investigated in an acute study, in which a single dose of EstroG-100™ of 0 (water for injection), 1,000, 2,000 or 4,000 mg/kg bw was administered via oral gavage to Sprague–Dawley rats (Crl:CD(SD)) (5 animals/sex per group) (Biotoxtech Co, 2010d). This study was conducted in compliance with KFDA principles on GLP and standards on toxicity studies (KFDA, 2009a,b). Compound-coloured stools and/or soft stools, which disappeared on day 2, were observed in the 4,000 mg/kg bw group only on day 1 after dosing. The authors concluded that the approximate lethal dose of EstroG-100™ was greater than 4,000 mg/kg under the conditions of this study.

Upon EFSA's request, the applicant provided acute oral toxicity studies on compounds from *A. gigas* Nakai: LD_{50} of decursin and decursinol angelate in rats was higher than 2,000 mg/kg bw (Kim et al., 2009c), and of esters and alcohols of cinnamic acid and cinnamyl alcohol, it was 1,520 mg/kg bw and higher in different species according to a review from Belsito et al. (2007).

3.8.4. Repeated dose toxicity studies

A subchronic 90-day oral toxicity study (Biotoxtech Co, 2015) on EstroG-100™ was provided by the applicant. This study was conducted in compliance with the OECD (1998) principles on GLP and the Japanese Guidelines on repeated dose toxicity studies. Sprague–Dawley rats (Crl:CD(SD)) (10 animals/sex per group; post-natal day: 6 weeks) were administered, via oral intubation, EstroG-100™ at doses of 0 (water vehicle), 500, 1,000, or 2,000 mg/kg bw per day for 90 consecutive days. These doses were selected based on the results from a repeated dose 28-day oral toxicity study in rats (TTC Inc., 2015) which reported a statistically significant increase in the relative liver weight in males and females for the two doses of EstroG-100™ tested (i.e. 1,000 and 3,000 mg/kg bw per day). A summary of the observations from the repeated dose 28- and 90-day oral toxicity studies is provided in Table 3.

The 90-day study reported coloured stools, which were probably caused by the study product, in eight males and four females in the 500 mg/kg bw groups and in all animals in the 1,000 and 2,000 mg/kg bw groups.

Hypersalivation was present sporadically in five males in the 1,000 mg/kg bw group and in 10 males and four females in the 2,000 mg/kg bw groups after dosing.

Statistically significant increases in total urinary excretion of potassium (K) and chloride (Cl) were noted in males in the 1,000 and 2,000 mg/kg bw groups as compared to the control group. Dose-related increase in reticulocytes, which was statistically significant only in the 1,000 mg group, was noted in females. A statistically significant decrease in erythrocyte count and statistically significant increases of mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) were noted in females in the 2,000 mg/kg bw group.

Statistically significant increases of albumin levels and A/G ratio were noted in males in the 2,000 mg/kg bw group, and statistically significant increases of total protein and albumin levels were noted in females in the 1,000 and 2,000 mg/kg bw groups.

Statistically significant increases in the absolute and relative liver weights were noted in females in the 1,000 and 2,000 mg groups. At necropsy, there was no observation of any macroscopic changes associated with the intervention. Microscopic examinations of tissues showed no between-group differences in the incidence of histopathological findings.

In addition, the applicant provided a subchronic 90-day oral toxicity study which was carried out with a product (EstroG-200) that contained 40.8% of EstroG-100™ together with minerals and vitamins (Moon et al., 2009). This study was conducted in compliance with the ICH guideline on chronic toxicity testing in animals and with the KFDA guideline on safety evaluation of drugs (ICH, 1998; KFDA, 2005). The results from this study have been summarised in Table 3.

Upon a request by EFSA for additional information, the applicant provided a publication on a subchronic 90-day oral toxicity study on a water extract of lyophilised *A. gigas* (AG) root (Yun et al., 2015), and two unpublished reports on subchronic 90-day oral toxicity studies on a micronised powder of *C. wilfordii* Hemsley and *P. umbrosa* Turcz. (TTC Co, 2013e; Biotoxtech Co, 2016) . The results from these studies have been summarised in Table 3.

The applicant also provided repeated dose oral toxicity studies on a water extract of lyophilised AG root at daily doses up to 2,000 mg/kg bw for 14 days (Yun et al., 2015); on decursin at daily doses up to 250 mg/kg bw for 4 weeks (Jiang et al., 2013); on decursin and decursinol angelate at daily doses up to 20 mg/kg bw for 30 days (Kim et al., 2009c). The applicant also provided a review on repeated-exposure oral toxicity studies on esters and alcohols of cinnamic acid and cinnamyl alcohol (Belsito et al., 2007). These studies did not show toxicity effects of the products investigated.

Table 3: Summary of repeated dose oral toxicity studies for the evaluation of the NF

Substance Method of preparation	**Dose levels tested Same dose level as EstroG-100™ (mg/kg bw), route**	**Species strain n/dose group**	**Lowest dose with effect (statistically significant) mg/kg bw day**	**Effect (affected sex)**
Subacute 28-day study				
EstroG-100™ **(TTC Inc., 2015)**	0, 1,000, 3,000 Suspension in water Oral gavage	Rat (SPF) Crl:CD(SD) 6 m, 6 f	1,000	Dose related: rel. liver weight ↑ (m,f), eosinophils ↓ (m) Not dose related: rel. heart weight ↑ (f) HCT ↑ (m), In urine: Na ↑ (m), Cl ↑ (m)
			3,000	Rel. kidney weight ↑ (f), Rel. salivary gland weights ↑ (f) Creatinine: ↓ (m) In urine: K ↓ (f)

Substance Method of preparation	Dose levels tested Same dose level as EstroG-100™ (mg/kg bw), route	Species strain n/dose group	Lowest dose with effect (statistically significant) mg/kg bw day	Effect (affected sex)
Subchronic 90-day studies				
EstroG-100™ **(Biotoxtech Co, 2015)**	0, 500, 1,000, 2,000 Suspension in water Oral gavage	Rat Sprague–Dawley (Crl:CD(SD)) 10 m, 10 f	500	Coloured stools Not dose related: rel. thymus weight ↓ (f), rel. spleen weight ↓ (f)
			1,000	Dose related: hypersalivation (m sporadically, at 2,000 m and f); in urine: K ↑ (m), Cl ↑ (m); albumin ↑ (f), total protein ↑ (f), reticulocytes ↑(f) (statistically significant only at 1,000), abs. + rel. liver weights ↑ (f)
			2,000	Erythrocytes ↓ (f), MCV ↑ (f), MCH ↑ (f), A/G ratio ↑ (m)
EstroG-200 (which includes 40.8% EstroG-100™, minerals and vitamins) **(Moon et al., 2009)**	0, 250, 500, 1,000 of EstroG-200 (which correspond to 0, 103, 206, 412 of EstroG-100™) Oral gavage Suspension in water	Rat Sprague Dawley 10 m, 10 f	103	Not dose related: creatine kinase ↓ (m), Ca ↑ (f)
			206	
			412	Ca ↓ (m), abs. brain weight ↓ (m)
Water extract of lyophilised *Angelica gigas* (35% in EstroG-100™) **(Yun et al., 2015)**	0, 125, 250, 500, 1,000, 2,000 (which correspond to 0, 357, 714, 1,428, 2,857, 5,700 of EstroG-100™) Oral gavage Suspension in water	Rat F344 10 m, 10 f	357	No effect
			714	Dose related: total protein ↑ (m), total cholesterol ↑ (m), albumin ↑ (m) Not clearly dose related: MCH ↓ (m)
			1,428	
			2,857	Dose related: Abs. liver weight ↑ (f) Not dose related: rel. liver weight ↑ (f)
			5,700	Abs. kidney weight ↑ (f), AST and ALT ↓ (m)
Micronised powder of *Cynanchum wilfordii* (32.5% in EstroG-100™) **(Biotoxtech Co, 2016)**	0, 150, 300, 600 (which correspond to 0, 462, 923, 1,846 of EstroG-100™) Oral gavage Suspension in water	Rat, Sprague–Dawley (Crl:CD(SD)) 10 m, 10 f	462	Not dose related: In urine: Na ↓ (m), K ↓ (m), Cl ↓ (m) In blood: GGT ↓ (m), Na ↓ (m) partial thromboplastin time ↓ (m), rel. brain weight ↓ (f)
			923	Dose related: rel. liver weight ↑ (f), rel. weight seminal vesicles ↑ (m), total cholesterol ↑ (f), Cl ↑ (m), Not dose related: total protein ↑ (f), abs. weight seminal vesicles ↑ (m);
			1,846	Abs. + rel. adrenal weight ↑ (f)
			1,846 4 weeks recovery	Total cholesterol ↑ (f) epididymis weight ↓ (m), neutrophils ↓ (f)
Micronised powder of *Phlomis umbrosa* (32.5% in EstroG-100™) **(TTC Co, 2013e)**	0, 300, 1,000 (which correspond to 0, 923, 3,077 of EstroG-100™) Oral gavage Suspension in water	Sprague–Dawley (Crl:CD(SD)) 6 m, 6 f	923	Not dose related: eosinophils ↑ (f) Inorganic P ↑ (m)
			3,077	Partial prothrombin time ↓ (m)

abs: absolute; bw: body weight; Cl: chloride; f: females; GGT: gamma glutamyl transpeptidase; HCT: haematocrit; K: potassium; m: males; MCH: mean corpuscular haemoglobin; MCV: mean corpuscular volume; Na: sodium; NF: novel food; rel.: relative.

The Panel notes that the studies provided, which have been summarised in Table 3, did not show effects on mortality or effects on histopathology up to the high dose levels tested. The changes observed are in absolute and relative organ weights, clinical chemical parameters, haematology and urine analysis. In the unpublished 90-day toxicity study with EstroG-100™ (Biotoxtech Co, 2015), no substance-related findings, besides coloured stools, are reported at the lowest dose level tested (500 mg/kg bw). At 1,000 mg/kg bw, several dose-related effects are reported: albumin and total protein levels are increased in females as well as absolute and relative liver weights, possibly reflecting increased protein synthesis in the liver. Increases in absolute liver weight, total protein and albumin, which were dose-related, were reported also in the study with a water extract of lyophilised *A. gigas* (Yun et al., 2015). Increase in relative liver weight, which was dose-related, was also reported in the study with a micronised powder of *C. wilfordii* Hemsley (Biotoxtech Co, 2016). In addition, in the unpublished 90-day toxicity study with EstroG-100™ (Biotoxtech Co, 2015), dose-related increases in reticulocytes were seen (20% at 1,000 mg/kg bw). This finding has to be seen as an adverse effect, which is supported by the fact that at the next dose level (i.e. 2,000 mg/kg bw), erythrocytes are decreased (7%) concomitant with an increase in MCV and MCH. Weight changes in seminal vesicles were reported in the subchronic toxicity study on a micronised powder of *C. wilfordii* Hemsley (at a dose corresponding to 923 mg/kg bw of EstroG-100™). In addition, weight changes were seen in the epididymides and the adrenals in the high-dose groups (which corresponded to a dose of 1,846 mg/kg bw of EstroG-100™). These observations point to possible endocrine effects, but only at high dose levels.

The Panel considers that the NOAEL derived from the subchronic 90-day oral toxicity study with EstroG-100™ (Biotoxtech Co, 2015), which was supported by observations in the other studies, is 500 mg/kg bw per day.

3.8.5. Human studies

The applicant provided two human intervention studies (Lee et al., 2005; Chang et al., 2012).

In the double-blind, placebo-controlled study by Lee et al. (2005), 48 women with 'diagnosed menopausal syndrome' (above 45 years of age), from a South Korean hospital, were randomised to consume either Estromon® (n = 24), which contained 40.81% of EstroG-100™ plus minerals and vitamins, or placebo (n = 24) for 12 months. The consumption of Estromon® corresponded to 514 mg/day of EstroG-100™.

This study investigated 'change of climacteric symptoms' and 'change in patients with climacteric symptoms at baseline' after 3 months of intervention.

This study reported a decrease in serum osteocalcin, alkaline phosphatase (ALP) and triglycerides levels in the intervention group as compared to the placebo group at 12 months. An increase in the femoral neck bone mineral density (BMD) and serum human growth hormone levels in the intervention group as compared to the placebo group at 12 months, were reported. There were no between-group changes for the other parameters reported (serum oestrogen, follicle stimulating hormone (FSH), serum total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), spinal bone density). No weight increase was observed.

The Panel notes that this study, which was carried out with a product containing minerals and vitamins in addition to EstroG-100™, did not investigate liver and haematological parameters. No adverse effects were reported in this study.

In the double-blind, placebo-controlled study by Chang et al. (2012), 64 women (White or African Americans) with menopausal symptoms (Kupperman menopause index (KMI) ≥ 20) were randomised to receive either EstroG-100™ (n = 31; 514 mg/day; age range 45–64 years) or placebo (n = 33; age range 42–70 years) for 12 weeks.

No adverse events were reported by participants. No between-group changes were observed in weight, body mass index (BMI), serum oestrogen, human growth hormone, osteocalcin, ALP, total cholesterol, LDL, HDL and triglycerides levels. There was a change in mean serum FSH level between the two groups, which was probably due to a decrease in the placebo group.

The Panel notes the short duration of this study and the absence of investigations of liver and haematological parameters.

The Panel considers that the two human studies with an intake of EstroG-100™ of 514 mg/day do not raise safety concerns with respect to the limited clinical chemical parameters investigated in relation to EstroG-100™. However, haematological and liver effects, which appeared in the toxicological

90-day repeated dose study, have not been investigated. The Panel notes that neither study was designed to assess the safety of the tested products.

3.8.6. Animal and *in vitro* studies on possible oestrogenic effects

The applicant provided animal and *in vitro* studies to address the potential oestrogenic effect of EstroG-100™.

3.8.6.1. Animal studies

The possible oestrogenic effect of EstroG-100™ was investigated in two studies with ovariectomised (OVX) rats (Kim et al., 2008; Lee et al., 2008).

In the study by Kim et al. (2008), 8-week-old Sprague–Dawley rats were ovariectomised and after 3 weeks recovery period they were divided into nine groups (seven rats each group): six groups of OVX rats received by gavage 73.5, 180, or 440 mg/kg bw per day of EstroG-100™ or Estromon® and the remaining three groups received distilled water (OVX control group, normal control group and sham control group) for 12 weeks.

There were no statistically significant differences in body weight, absolute uterus, liver and kidney weights between the EstroG-100™/Estromon® groups and OVX control group. There was a statistically significant increase in serum insulin-like growth factor 1 (IGF-1) levels in two EstroG-100™ groups (i.e. 180 and 440 mg/kg bw) ($p < 0.05$). All the EstroG-100™ groups showed a statistically significant decrease in the serum osteocalcin levels ($p < 0.05$). A statistically significant increase in the femoral BMD was found in the EstroG-100™ and Estromon® groups ($p < 0.05$). In particular, all EstroG-100™ groups showed a significant dose-dependent increase in bone density ($p < 0.05$).

In the study by Lee et al. (2008), 51 forty-week-old Sprague–Dawley rats were ovariectomised and after 3 weeks recovery they were separated into six groups which were administered by gavage isoflavones, EstroG-100™ (at doses of 100 mg/kg bw or 500 mg/kg bw per day), Estromon® 100 mg/kg bw per day, or distilled water (sham and OVX control groups) for 12 weeks.

There was no statistically significant difference in body and uterus weight between the EstroG-100™/Estromon® groups and OVX control group. A statistically significant increase in femoral BMD was observed ($p < 0.05$) in the 100 mg/kg bw EstroG-100™ group as compared to the OVX control group.

The Panel notes that OVX rats were exposed to interventions for 12 weeks, which is a longer period than the 3-day period requested as minimum in the uterotrophic assay by the OECD Guideline test N. 440 (OECD, 2007). The longer duration would allow detecting oestrogenic substances with higher sensitivity. The relevant endpoint (i.e. increase in uterine weight) was unchanged, indicating that there is a lack of oestrogenic activity.

3.8.6.2. *In vitro* studies

Binding affinity of EstroG-100™ to the oestrogen α and β receptors (ERα and ERβ) was investigated in an *in vitro* study, where 17-β estradiol was used as a positive control (Research Institute of Veterinary Medicine, 2008). There was no increase in absorbance, which was associated with binding affinity to the oestrogen receptors, with EstroG-100™. The authors concluded that EstroG-100™ has no affinity to both ERα and ERβ. The Panel notes that competing with oestrogen for receptor binding was not investigated in this study.

The effect of EstroG-100™ and hot-water extracts of each individual root, at concentrations up to 1,000 μg/mL was investigated in the oestrogen-dependent Michigan Cancer Foundation-7 human breast cancer cells (MCF-7) (Lee and Jeong, 2007). This study reported a dose-dependent growth inhibition of the MCF-7 cells at high doses of EstroG-100™ as well as of *A. gigas* Nakai. The Panel notes that this study did not include a positive control with oestrogen. Therefore, no conclusions can be drawn for the test system used in this study.

Lee et al. (2008) investigated the effect of EstroG-100™, and the extracts of each individual root on ALP activity in Ishikawa and SaOS-2 cells. In these cell lines, ALP activity and expression of mRNA of ALP can be stimulated by oestrogen. EstroG-100™ was superior in ALP induction as compared to the individual roots. But all extracts were less active than 10^{-12} M oestrogen.

The Panel notes that EstroG-100™ does not exhibit classical oestrogenic effects. This view is based on the absence of changes in sex hormone levels in menopausal women, the absence of reactivity of the uterus in OVX rats and lack of oestrogen binding in receptor binding assays.

3.9. Allergenicity

According to the applicant, there is no history of allergenicity associated with the use of the three plants which constitute EstroG-100™.

As outlined in the proposed specifications (Table 1), the protein content in EstroG-100™ ranges between 70 and 170 mg/g.

A MS commented on the risk of allergic reactions in individuals with celery allergy due to the fact that celery and *A. gigas* Nakai belong to the same botanical family (i.e. Apiaceae). Since the risk of allergic reactions in subjects with known celery allergy cannot be excluded, the applicant proposed to label that EstroG-100™ should not be consumed by individuals with known celery allergy.

Based on the limited data available, the Panel considers that allergic reactions to the NF cannot be ruled out, but the likelihood is low.

4. Discussion

The NF is EstroG-100™, an extract of a mixture of three herbal roots: *C. wilfordii* Hemsley (32.5%), *P. umbrosa* Turcz. (32.5%) and *Angelica gigas* Nakai (35%). The mixture of the three herbal roots is hot-water extracted and concentrated, and dried by spray drying.

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

The applicant intends to use EstroG-100™ in food supplements (as tablets, powder, capsules, softgel or gelcaps). The proposed maximum intake level is 514 mg/day of EstroG-100™. The target population is post-menopausal women.

Taking into account the composition of the NF and the intended use levels, the Panel considers that the NF does not have a nutritionally relevant role in the human diet and that the consumption of the NF is not nutritionally disadvantageous.

A bacterial reverse mutation test, an *in vitro* chromosome aberration test and an *in vivo* micronucleus test on the NF did not show indications of mutagenicity or chromosome aberrations for the NF. The same genotoxicity tests were performed on the single plants which constitute EstroG-100™ and did not show indications of mutagenicity or chromosome aberrations for the plants investigated. The Panel considers that the information provided does not raise safety concerns as regards the genotoxicity of the NF.

In a subchronic 90-day repeated dose oral toxicity study, EstroG-100™ was administered to Sprague–Dawley rats, via oral intubation, at doses of 0, 500, 1,000 or 2,000 mg/kg bw per day. This study reported dose-related effects at the dose of 1,000 mg/kg bw per day (e.g. increase in albumin, total protein levels, reticulocytes, absolute and relative liver weights). Some of these effects were also observed in a repeated dose 28-day oral toxicity study with EstroG-100™ and repeated dose 90-day oral toxicity studies with the single plants which constitute EstroG-100™. The Panel considers that the NOAEL derived from the subchronic 90-day oral toxicity study with EstroG-100™, which was supported by observations in the other studies, is 500 mg/kg bw per day.

Two double-blind, placebo-controlled studies in women were provided by the applicant. One study, which lasted 12 months, was carried out with Estromon®, which contained minerals and vitamins in addition to EstroG-100™. The second study, which lasted 12 weeks, was carried out with EstroG-100™. Both human studies did not raise safety concerns with respect to the limited clinical chemical parameters investigated. However, haematological and liver effects, which appeared in the toxicological 90-day repeated dose study, have not been investigated in these studies. The Panel notes that neither study was designed to assess the safety of the tested products.

Based on the observations from animal and *in vitro* studies on potential oestrogenic effect of the NF, the Panel considers that EstroG-100™ does not exhibit classical oestrogenic effects.

Taking into account the NOAEL of 500 mg/kg bw per day and the proposed maximum intake level of 514 mg/day for the NF, which corresponds to 7.3 mg/kg bw per day for an adult of 70 kg bw (default body weight value as indicated by the EFSA Scientific Committee (2012)), the Panel considers that the margin of safety of 68 (i.e. ratio between the NOAEL and the proposed maximum intake level), is not sufficient. Based on the absence of chronic toxicity data, an increase in effects with exposure duration in toxicity studies (effects on liver (weight, albumin, total protein) in the 90-day repeated dose study as compared to the 28-day repeated dose study), and the absence of investigations of liver parameters and haematology in human studies, the Panel applies the uncertainty

factor of 200 to derive the maximum safe intake level for the NF (EFSA Scientific Committee, 2012). Thus, by applying the uncertainty factor of 200 to the NOAEL, the Panel derives the maximum intake level of 2.5 mg/kg bw per day for the NF, which corresponds to 175 mg/day for an adult of 70 kg bw.

5. Conclusions

The Panel concludes that the NF, EstroG-100™, is safe for the use in food supplements at the maximum intake level of 175 mg/day for an adult of 70 kg bw.

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 EstroG-100™ as a novel food ingredient. SANTE/E6/SH/ks Ref. Ares(2015)1664994, dated 20 April 2015.
2) Dossier 'Application for the approval of an extract of mixture of three herbal roots (EstroG-100™) for use as a novel food ingredient' received by EFSA on 23 April 2015, which was submitted by Naturalendo Tech Co., Ltd.
3) On 30 June 2015, during the validation process of the application, EFSA sent a request to the applicant to provide missing information.
4) Upon a request by EFSA for missing information, on 30 June 2015, EFSA received the missing information as submitted by the applicant.
5) Initial assessment report carried out by the Food Safety Authority of Ireland: 'Safety assessment of an extract of herbal roots (EstroG-100™).'
6) Member States' comments and objections.
7) Response by the applicant to the initial assessment report and the Member States' comments and objections.
8) Additional data were provided by the applicant on 18 December 2015 and 15 April 2016.

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Abbreviations

AG	*Angelica gigas*
ALP	alkaline phosphatase
BMD	bone mineral density
BMI	body mass index
bw	body weight
CHL	Chinese hamster Lung
FSH	follicle stimulating hormone
GGT	gamma glutamyl transpeptidase
GLP	good laboratory practice
GMP	good manufacturing practice
HACCP	hazard-analysis and critical control points
HCT	haematocrit
HDL	high-density lipoprotein
HPLC	high-performance liquid chromatography
ICH	International Conference on Harmonisation
KFC	Korean Food Code
KFDA	Korea Food and Drug Administration
KMI	Kupperman menopause index
IGF-1	insulin-like growth factor 1
LD_{50}	lethal dose, median
LDL	low-density lipoprotein
MCF-7	Michigan Cancer Foundation-7 human breast cancer cells
MCH	mean corpuscular haemoglobin
MCV	mean corpuscular volume
MNPCE	micronucleated polychromatic erythrocytes
MS	Member State
NF	novel food
NMT	not more than
NOAEL	no-observed-adverse effect level
OECD	Organisation for Economic Co-operation and Development
OVX	ovariectomised
PCE	polychromatic erythrocytes