Application for the Approval of Tongkat Ali Root Extract as a Novel Food

Pursuant to

Regulation (EC) No 258/97 of the European Parliament and of the Council of 27th January 1997 Concerning Novel Foods and Novel Food Ingredients

NON-CONFIDENTIAL DOSSIER

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Table of Contents

Page

List of Figures and Tables

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ADMINISTRATIVE DATA

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EXECUTIVE SUMMARY

Approval is sought under Regulation (EC) No 258/97 of the European Parliament and of the Council of 27th January 1997 concerning novel foods and novel food ingredients, for the dried, ground root chips of *Eurycoma longifolia* (hereinafter referred to as Tongkat Ali Root Extract) as an ingredient in conventional foods and food supplements (European Parliament and the Council of the European Union, 1997).

Tongkat Ali Root Extract is a standardised water extract prepared from the dried ground root chips of *E. longifolia*. The source plant is not genetically modified. As such, this ingredient falls under category (e) of Article 1(2) or Regulation (EC) No 258/97: "foods and food ingredients consisting of or isolated from plants and food ingredients isolated from animals, except for foods and food ingredients obtained by traditional propagating and breeding practices and which have a history of safe food use".

Biotropics' Tongkat Ali Root Extract is standardised to total polysaccharide (greater than 30%), total protein (greater than 22%), total glycosaponin (greater than 40%), and eurycomanone content (0.8 to 1.5%), consistent with the specifications established in the Malaysian Standard for this extract (MS 24089:2011). Furthermore, the characteristic component of the ingredient, eurycomanone, is used as a marker for high-performance liquid chromatographic characterisation.

Appropriate product specifications for identity and potential contaminants have been established for this ingredient. The results of batch analyses indicate that the manufacturing process produces a consistent product meeting the product specifications. The product is free from heavy metal, microbial, pesticide, and aflatoxin contamination. Furthermore, the results of stability studies indicate that Tongkat Ali Root Extract is stable beyond its intended shelf life of 36 months.

Tongkat Ali Root Extract is manufactured according to Good Manufacturing Practice and is compliant with ISO 22000:2005 and Hazard Analysis and Critical Control Points (HACCP) principles. Tongkat Ali Root Extract is produced by water extraction, followed by concentration by evaporation, heat sterilisation, and either freeze- or spray- drying. The ingredient is then ground/milled into a fine powder and double-packaged in plastic / aluminium bags. No organic solvents and no excipients are used during the production process. These methods are typical of the food industry and are not anticipated to result in any toxicological, nutritional, or microbiological hazards. The raw materials and processing aids used in the production of Tongkat Ali Root Extract have not been genetically modified.

Consumption of *E. longifolia* is prevalent in South-East Asian countries including Malaysia, Indonesia, and Vietnam. In particular, the root extract of *E. longifolia* has been traditionally prepared by boiling and consumed as a tonic for aphrodisiac effects, virility and energy in men. In women, the root extract is common in post-partum and confinement concoctions for recovery and to regain strength. *E. longifolia* is most popularly consumed as a beverage (coffee, energy drink, tonic) and marketed by brands like Power Root, Ali Cafe, Nestle, CNI

and many other companies with many products registered with GNPD (Global New Products Database - Mintel Group, 2013). Additionally, *E. longifolia* extracts have been marketed in the United States for 13 years with no reports of adverse events.

Tongkat Ali Root Extract is proposed for use as an ingredient in beverages (coffee, sports and energy drinks, tea-based beverages), chocolate and confectionary (chocolate bars, candies), cereal bars, products for special nutritional use (nutrition and energy bars) and food supplements (including capsule form, tablet form, and liquid form). The proposed use level in conventional food matrices is from 50 to 75 mg per serving and the proposed use level in supplement forms are 200 mg/day.

The potential intakes of Tongkat Ali Root Extract from its proposed uses in conventional foods were estimated using consumption data from the European Food Safety Authority (EFSA) Comprehensive Food Consumption Database utilising the Food Additive Intake Model (FAIM) tool. Based on the maximum proposed use of the ingredient, which was applied across the broad food categories described in the FAIM tool, the highest mean and heavy-level intakes on a per kilogram body weight basis in the target population of adults were 0.8 to 5.9 mg/kg body weight/day and 3.3 to 12.1 mg/kg body weight/day, respectively. Among the non-target demographic that may be unintentionally exposed to Tongkat Ali Root Extract, the worst-case heavy-level intakes of the ingredient were estimated to be 3.0 to 11.1 mg/kg body weight/day in adolescents. Using a more refined intake assessment with data from the United Kingdom National Diet and Nutrition Survey (UK NDNS), a mean and 95th percentile intake was calculated assuming Tongkat Ali Root Extract was used at the maximum use level in its proposed food use categories (Department of Health, 2014; UKDA, 2014). The mean and 95th percentile intakes among consumers of foods in which the ingredient is proposed for use was 56.4 and 165.9 mg/day, respectively (equivalent to 0.75 and 2.26 mg/kg body weight/day) in the adult target population. The results of the assessment also indicate that the highest worst-case 95th percentile Tongkat Ali Root Extract intake for the non-target teenager population group was 150.1 mg/day (equivalent to 3.01 mg/kg body weight/day).

In addition to the proposed uses of the ingredient in conventional foods, it is noted that Tongkat Ali Root Extract also is intended to be an ingredient in food supplements marketed to adult men and women. The proposed use level of Tongkat Ali Root Extract is 200 mg per daily dose. Food supplements containing Tongkat Ali Root Extract would be consumed as an alternative source of Tongkat Ali Root Extract in conventional food forms and will be conspicuously labelled as such; therefore, it is not expected that individuals will consume both supplements and foods containing Tongkat Ali Root Extract.

Tongkat Ali Root Extract is not nutritionally equivalent to other foods and is not intended to replace other foods currently on the market. Tongkat Ali Root Extract is not anticipated to impact the quality of the diet nor play any role in the diet. The proximate characteristics of the ingredient have been characterised: the caloric content is in the region of 350 to 360 kcal per 100 g, the carbohydrate content is in the range of 73 to 85 g per 100 g, and protein is in

the range of 4 to 12 g per 100 g (by total nitrogen method). Tongkat Ali Root Extract does not contain appreciable levels of fatty acids.

The toxicological properties of Tongkat Ali Root Extract have been evaluated through traditional pre-clinical safety studies, as well as several human studies. Using eurycomanone as a marker, Tongkat Ali Root Extract is expected to have an oral bioavailability of approximately 10.5%. Tongkat Ali Root Extract is not acutely toxic, as demonstrated by its oral median lethal dose value of greater than 2,000 mg/kg body weight. The results of a 28-day, 90-day, and 1-year repeat dose oral toxicity studies in rats have indicated that no adverse effects in standard toxicological parameters are observed following oral administration of 250, 500, or 1,000 mg/kg body weight/day. As such, the no-observed-adverse-effect level for oral toxicity has been established as 1,000 mg/kg body weight/day, the highest dose tested. Based on the results of a reproductive and developmental toxicity screening study, Tongkat Ali Root Extract is not anticipated to have adverse effects on reproductive or developmental endpoints. The results of a bacterial reverse mutation assay, an *in vitro* mammalian cell gene mutation assay, and an *in vivo* mouse erythrocyte micronucleus assay indicate the ingredient is not genotoxic.

The results of studies in which Biotropics' Tongkat Ali Root Extract has been provided to human subjects have demonstrated that consumption of the ingredient at doses of up to 400 mg/day for up to 2 months have not been associated with adverse outcomes nor deleterious effects in haematological, blood biochemical, hormone, immune, or quality of life parameters. Furthermore, there is no evidence of adverse health effects (including the development of sensitivities or intolerances) in other studies investigating efficacy parameters following consumption of Tongkat Ali Root Extract for up to 12 weeks.

Taken together, the scientific evidence presented demonstrates that Biotropics' Tongkat Ali Root Extract ingredient is not anticipated to pose any adverse effects on human health under the proposed conditions of use as a novel food ingredient in conventional foods and food supplements.

INTRODUCTION

Biotropics Malaysia Berhad proposes to market a standardised water extract prepared from the dried ground root chips of *Eurycoma longifolia* (Tongkat Ali Root Extract) as an ingredient in conventional foods and food supplements in Europe. Approval is sought for Tongkat Ali Root Extract under Regulation (EC) No 258/97 of the European Parliament and of the Council of 27th January 1997 concerning novel foods and novel food ingredients (hereafter referred to as EC 258/97). Accordingly, this submission has been prepared pursuant to the *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* (hereafter referred to as the Commission Recommendation of 1997) (European Parliament and the Council of the European Union, 1997).

Article 1(2.) of EC 258/97 states that the regulation "…shall apply to the placing on the market within the Community of foods and food ingredients which have not hitherto been used for human consumption to a significant degree within the Community and which fall under the following categories…(e) foods and food ingredients consisting of or isolated from plants and food ingredients isolated from animals, except for foods and food ingredients obtained by traditional propagating and breeding practices and which have a history of safe food use" (European Parliament and the Council of the European Union, 1997). Tongkat Ali Root Extract is thus considered a novel food/food ingredient within this category.

Section 4 of the Commission Recommendation of 1997 outlines recommendations made by the Scientific Committee on Food (SCF) related to the "Scientific Classification of Novel Foods for the Assessment of Wholesomeness", which facilitates the safety and nutritional evaluation of a given novel food/food ingredient. Of the 6 classes identified, Tongkat Ali Root Extract would be classified in Class 2 as a "complex NF from non-GM source", since the preparation of the ingredient is developed by conventional techniques, and with no use of genetic modification. Tongkat Ali Root Extract does not have a significant history of use within the Community. Accordingly, the ingredient would be further allocated under Sub-Class 2.2: "the source of the novel food has no history of food use in the Community". The essential information requirements corresponding with this classification are outlined in a detailed list below, and are expanded upon in separate sections throughout the document, forming the basis of the application (Recommendation 97/618/EC - Commission of the European Communities, 1997).

I	Specification of the Novel Food
II	Effect of the Production Process Applied to the Novel Food
III	History of the Organism Used as the Source of the Novel Food
IV-VIII	Not Applicable
IX	Anticipated Intake/Extent of Use of the Novel Food
X	Information from Previous Human Exposure to the Novel Food or Its Source
XI	Nutritional Information on the Novel Food

XII Microbiological Information on the Novel Food
XIII Toxicological Information on the Novel Food

For each category (I through XIII), structured schemes have been developed by the SCF, which consist of a decision-tree-like set of questions designed to elicit sufficient data for a comprehensive safety and nutritional evaluation of the novel food. Section X has been included for discussion, as Tongkat Ali Root Extract has been previously marketed as a food ingredient in jurisdictions outside of the European Union. As outlined below in Sections I through XIII, the required questions are identified and subsequently addressed with the appropriate data.

I SPECIFICATIONS FOR TONGKAT ALI ROOT EXTRACT

Based on the SCF guidelines, the following questions must be answered:

- “Is appropriate analytical information available on potentially toxic inherent constituents, external contaminants and nutrients?”
- “Is the information representative of the novel food when produced on a commercial scale?”
- “Is there an appropriate specification (including species, taxon *etc.* for living organisms) to ensure that the novel food marketed is the same as that evaluated?”

These questions have been addressed collectively in Sections I.A through I.E.

I.A Identity

I.A.1 Common Name or Usual Name

Tongkat Ali Root Extract

I.A.2 Chemical Abstract Service (CAS) Number

Not available for extract.

The characteristic compound, erycomanone, is identified by the CAS number 84633-29-4.

I.A.3 Description

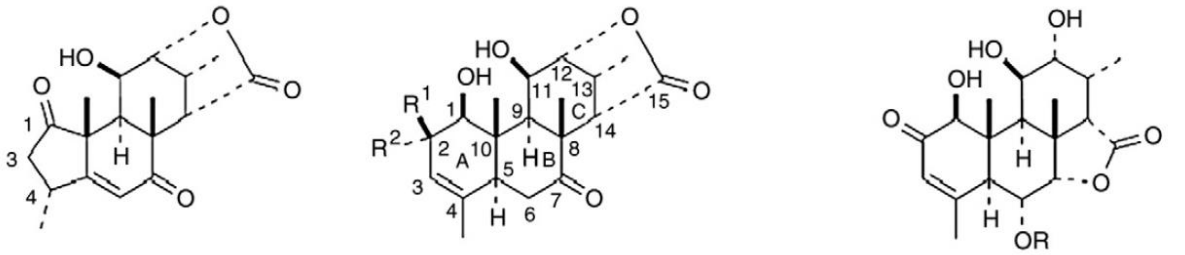
Tongkat Ali Root Extract is a standardised water extract prepared from the dried grounded root chips of *Eurycoma longifolia.*

I.A.4 Chemical Composition

The Tongkat Ali Root Extract is standardised to total polysaccharide (30 to 55%), total protein (22 to 45%), total glycosaponin (40 to 65%), and eurycomanone content (0.8 to 1.5%), consistent with the specifications established in the Malaysian Standard for this extract. It is also shown to contain a characteristic peptide (4.3 kDa) (see Figures below), which has been associated with the extract's physiological effects. More comprehensive proximate analysis data are presented in Table I.C.2.

The characteristic component of Tongkat Ali Root Extract is eurycomanone (a quassinoid), comprising between 0.8 to 1.5% of the extract. The major eurycomanone species (eurycolactone, eurycolactone E, and eurycolactone F) are presented in Figure I.A.4-1.

Figure I.A.4-1 The major bioactive quassinoids (eurycomanone) in Tongkat Ali Root Extract



Eurycolactone | Eurycolactone E (R^1= H, R^2 = OH) | Eurycolactone F (R = Ac)

Adapted from Bhat and Karim, 2010.

Tongkat Ali Root Extract is further characterised by chromatographic methods using eurycomanone as a marker. The high-performance liquid chromatography chromatogram of the eurycomanone standard and a sample of Tongkat Ali Root Extract are presented in Figure I.A.4-2. The Tongkat Ali Root Extract may be further characterised by matrix-assisted laser desorption/ionisation (MALDI) with time of flight (TOF) detection using α-cyano-4-hydroxycinnamic acid as a matrix (see Figure I.A.4-3).

Figure I.A.4-2 High-performance liquid chromatography (HPLC) chromatograph of Eurycomanone and Tongkat Ali Root Extract

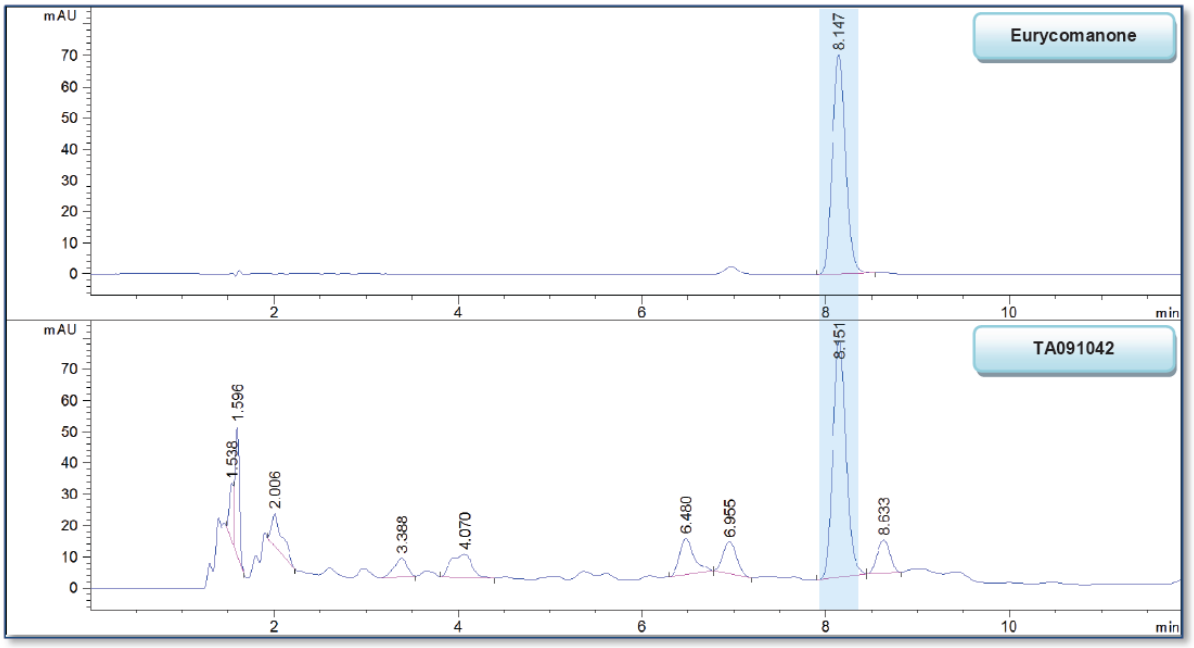
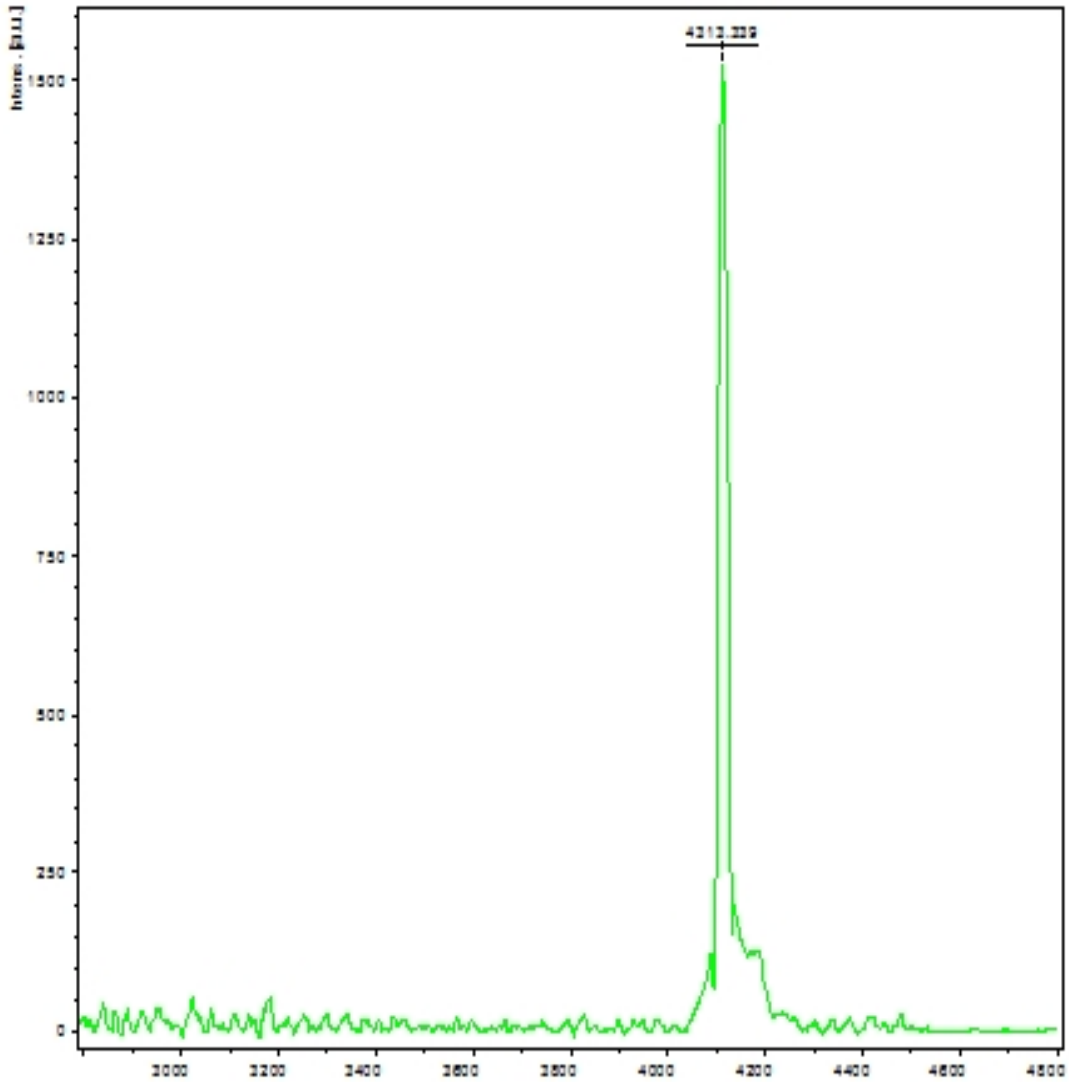


Figure I.A.4-3 MALDI-TOF with α-cyano-4-hydroxycinnamic Acid (HCCA) as the matrix



I.B Product Specifications

The product specifications established for Tongkat Ali Root Extract are presented in Table I.B-1. Specifications were based on the standardisation parameters outlined in *The Malaysian Standard's* phytopharmaceutical aspect of dried water extract from tongkat ali roots (Department of Standards Malaysia, 2011).

Table I.B-1 Specifications Established for Tongkat Ali Root Extract		
Parameter	**Specifications**	**Methods**
Organoleptic		
Colour	Light brown to brown	Standard Operating Procedure PD/SOP/02
Odour	Characteristic	Standard Operating Procedure PD/SOP/02
Flavour	Bitter	Standard Operating Procedure PD/SOP/02
Form/texture	Fine powder	Standard Operating Procedure PD/SOP/02
Extraneous material	Free from foreign matter	Standard Operating Procedure PD/SOP/02
Physical Characteristics		
Moisture content (%)	< 8.0	Loss on drying PD/SOP/13
Average mesh size	90% smaller than 100 mesh	Standard Operating Procedure PD/SOP/12
Bioactive content		
Eurycomanone (%)	0.8 to 1.5	Standard Test Method TM/R&D/001
Total protein (%)	22 to 45	Lowry Method PD/SOP/09
Total polysaccharide (%)	30 to 55	Anthrone reagent PD/SOP/10
Glycosaponin (%)	40 to 65	Gravimetric method PD/SOP/11
Heavy Metals		
Lead (mg/kg)	< 2.0	Ph. Eu. 2.4.27
Mercury (mg/kg)	< 0.05	Ph. Eu. 2.4.27
Arsenic (mg/kg)	< 1.0	Ph. Eu. 2.4.27
Cadmium (mg/kg)	< 0.3	Ph. Eu. 2.4.27
Microbial Specifications		
Total bacteria count (CFU/g)	< 10,000	Ph. Eu. 2.6.12
Yeast and mould (CFU/g)	< 100	Ph. Eu. 2.6.12
Salmonella (/10g)	Absent	Ph. Eu. 2.6.12
Escherichia coli (/1g)	Absent	Ph. Eu. 2.6.12
Staphylococcus aureus (/1g)	Absent	Ph. Eu. 2.6.12
Bile-tolerant gram-negative bacteria (bacteria/g)	< 100	Ph. Eu. 2.6.12

Abbreviations: CFU = colony forming units; Ph. Eu. = European Pharmacopeia.

I.C Batch Analyses

I.C.1 Product Specifications

The results of analyses of 3 non-consecutive batches of Tongkat Ali Root Extract are presented in Table I.C-1. The results indicate that the manufacturing process for Tongkat Ali Root Extract yields a consistent product that complies with established specifications.

Table I.C-1 Analyses for 3 Non-consecutive Batches of Tongkat Ali Root Extract				
Parameter	**Specifications**	**TA 130511**	**TA 130831**	**TA 130935**
Organoleptic				
Colour	Light brown	Complies	Complies	Complies
Odour	Characteristic	Complies	Complies	Complies
Flavour	Bitter	Complies	Complies	Complies
Form/texture	Fine powder	Complies	Complies	Complies

Table I.C-1 Analyses for 3 Non-consecutive Batches of Tongkat Ali Root Extract				
Parameter	**Specifications**	**TA 130511**	**TA 130831**	**TA 130935**
Extraneous material	Free from foreign matter	Complies	Complies	Complies
Physical Characteristics				
Moisture content (%)	< 8.0	2.11	3.45	2.43
Average mesh size	90% smaller than 100 mesh	Complies	Complies	Complies
Bioactive content				
Eurycomanone (%)	0.8 to 1.5	0.91	0.87	0.81
Total protein (%)	22 to 45	27.6	29.9	30.8
Total polysaccharide (%)	30 to 55	33.5	34.7	36.5
Glycosaponin (%)	40 to 65	47.2	49.3	51.5
Heavy Metals				
Lead (mg/kg)	< 2.0	< 0.10	< 0.10	< 0.10
Mercury (mg/kg)	< 0.05	< 0.01	< 0.01	< 0.01
Arsenic (mg/kg)	< 1.0	< 0.01	< 0.01	0.04
Cadmium (mg/kg)	< 0.3	< 0.01	< 0.01	< 0.01
Microbial Specifications				
Total bacteria count (CFU/g)	< 10,000	< 10	< 10	< 10
Yeast and mould (CFU/g)	< 100	< 10	< 10	< 10
Salmonella (/10g)	Absent	Absent	Absent	Absent
Escherichia coli (/1g)	Absent	Absent	Absent	Absent
Staphylococcus aureus (/1g)	Absent	Absent	Absent	Absent
Bile-tolerant gram-negative bacteria (bacteria/g)	< 100	< 10	< 10	< 10

Abbreviations: CFU = colony forming units.

I.C.2 Proximate Analysis

Proximate analyses also have been conducted on samples of Tongkat Ali Root Extract and are presented in Table I.C.2-1. The ingredient contains negligible levels of fat and is comprised mainly of carbohydrates and proteins.

Table I.C.2-1 Proximate Analysis on Tongkat Ali Root Extract				
Parameter	**TA 141043**	**TA 141049**	**TA 06111450**	**Methods**
Energy (kcal/100g)	351	349	363	Based on Method of Analysis for Nutrition Labelling - AOAC, 1993
Energy from fat (kcal/100 g)	0	0	0	Based on Method of Analysis for Nutrition Labelling - AOAC, 1993
Total Fat (g/100g)	< 0.10	< 0.10	< 0.10	Based on Pearson's Chemical Analysis of Foods, 7th Ed, 1976
Carbohydrate (g/100g)	73.6	74.4	84.6	Based on Method of Analysis for Nutrition Labelling – AOAC, 1993
Total dietary fibre (g/100g)	4.12	3.0	2.73	AOAC 985.29 (Enzymatic-Gravimetric Method)
Protein (g/100g)	12.2	11.3	4.70	Based on AOAC 991.20 (Total Nitrogen Method)

I.D Contaminants

Specifications for heavy metal and microbial contamination are established to ensure the absence of these substances in Tongkat Ali Root Extract. Furthermore, Tongkat Ali Root Extract has been analysed for the presence of organochlorine and organophosphorus pesticides and aflatoxins. The results of these analyses confirm that the ingredient is free of these contaminants.

I.E Stability

The results of real-time and accelerated stability testing conducted on 2 independent batches of Tongkat Ali Root Extract are summarised in Table I.E-1. The results indicate that Tongkat Ali Root Extract conforms to the physical and microbial specifications established for the ingredient for at least its recommended shelf life of 24 months and up to 36 months.

Table I.E-1 Results of Real-Time and Accelerated Stability Testing on Tongkat Ali Root Extract

Parameter	Spec.	Time (Months)									
		0	**1**	**2**	**3**	**6**	**9**	**12**	**18**	**24**	**36**
Real-time (30°C, relative humidity of 75%)											
Appearance and colour	Light brown	Conforms	-	-	Conforms	Conforms	Conforms	Conforms	Conforms	-	-
Moisture (%)	< 8	4.46	-	-	4.76	4.32	4.25	4.39	4.62	4.74	4.38
Total protein (%)	> 22.0	30.20	-	-	23.60	22.40	28.40	28.40	30.30	38.60	25.10
Total glycosaponin (%)	> 35	40.90	-	-	38.20	45.20	49.30	48.80	46.80	47.10	44.30
Eurycomanone (%)	0.8 to 1.5	0.93	-	-	31.00	31.40	31.50	30.30	34.50	33.30	30.30
Lead (mg/kg)	< 10.0	< 1.0	-	-	-	-	-	-	-	-	-
Arsenic (mg/kg)	< 5.0	< 1.0	-	-	-	-	-	-	-	-	-
Mercury (mg/kg)	< 0.5	< 0.05	-	-	-	-	-	-	-	-	-
Cadmium (mg/kg)	< 0.3	< 0.2	-	-	-	-	-	-	-	-	-
Total aerobic microbial count (CFU/g)	< 10,000	< 10	-	-	-	< 10	-	< 10	-	< 10	< 10
Total yeast and mould count (CFU/g)	< 100	< 10	-	-	-	< 10	-	< 10	-	< 10	< 10
Salmonella (/10 g)	Absent	Absent	-	-	-	Absent	-	Absent	-	Absent	Absent
Escherichia coli (/1 g)	Absent	Absent	-	-	-	Absent	-	Absent	-	Absent	Absent
Staphylococcus aureus (/1 g)	Absent	Absent	-	-	-	Absent	-	Absent	-	Absent	Absent
Enterobacteriaceae (/g)	< 100	< 10	-	-	-	< 10	-	< 10	-	< 10	< 10
Accelerated (40°C, relative humidity of 75%)											
Appearance and colour	Light brown	Conforms	Conforms	Conforms	Conforms	Conforms	-	-	-	-	-
Moisture (%)	< 8	4.46	4.13	4.75	4.23	4.73	-	-	-	-	-
Total protein (%)	22.0 to 45	30.20	29.37	28.80	24.50	25.50	-	-	-	-	-
Total glycosaponin (%)	35 to 65	40.90	40.30	38.86	40.60	42.10	-	-	-	-	-
Eurycomanone (%)	0.8 to 1.5	0.93	1.01	0.91	0.92	0.81	-	-	-	-	-

Table I.E-1 Results of Real-Time and Accelerated Stability Testing on Tongkat Ali Root Extract

Parameter	Spec.	Time (Months)									
		0	**1**	**2**	**3**	**6**	**9**	**12**	**18**	**24**	**36**
Lead (mg/kg)	< 10.0	< 1.0	-	-	-	-	-	-	-	-	-
Arsenic (mg/kg)	< 5.0	< 1.0	-	-	-	-	-	-	-	-	-
Mercury (mg/kg)	< 0.5	< 0.05	-	-	-	-	-	-	-	-	-
Cadmium (mg/kg)	< 0.3	< 0.2	-	-	-	-	-	-	-	-	-
Total aerobic microbial count (CFU/g)	< 10,000	< 10	-	-	-	< 10	-	-	-	-	-
Total yeast and mould count (CFU/g)	< 100	< 10	-	-	-	< 10	-	-	-	-	-
Salmonella (/10 g)	Absent	Absent	-	-	-	Absent	-	-	-	-	-
Escherichia coli (/1 g)	Absent	Absent	-	-	-	Absent	-	-	-	-	-
Staphylococcus aureus (/1 g)	Absent	Absent	-	-	-	Absent	-	-	-	-	-
Enterobacteriaceae (/g)	< 100	< 10	-	-	-	< 10	-	-	-	-	-

Abbreviation: - = analysis not available; CFU = colony forming units; Spec. = specification.
Results for the representative batch TA 090620 are presented herein.

II EFFECT OF THE PRODUCTION PROCESS APPLIED TO THE INGREDIENT

Based on the SCF guidelines, the following questions must be addressed:

- “Does the novel food undergo a production process?”
- “Is there a history of use of the production process for the food?” If no, “does the process result in a significant change in the composition or structure of the novel food compared to its traditional counterpart?”
- “Is information available to enable identification of the possible toxicological, nutritional and microbiological hazards arising from use of the process?”
- “Are the means identified for controlling the process to ensure that the novel food complies with its specification?”
- “Has the process the potential to alter the levels in the novel food of substances with an adverse effect on public health?”
- “After processing is the novel food likely to contain microorganisms of adverse public health significance?”

These questions have been addressed in Sections II.A through II.F.

II.A Production Process

The manufacturing process of Tongkat Ali Root Extract is conducted according to Good Manufacturing Practice (GMP) and is compliant with ISO 22000:2005 and Hazard Analysis and Critical Control Points (HACCP) principles. Dried woodchips of Tongkat Ali root are subject to water extraction and filtration, followed by evaporation and sterilisation. The concentrated extract is then dried and homogenised.

II.B History of Use of the Production Process

Tongkat Ali Root Extract is prepared using traditional drying, water extraction, and grinding methods. These methods are typical of the food industry.

II.C Identification of the Potential Toxicological and Nutritional Hazards Arising from the Production Process

It is not anticipated that any toxicological, nutritional, or microbiological hazards will arise from the production process. The raw material (*E. longifolia* root chips) is checked for microbiological quality and foreign material and must pass specifications before its use as the source material for the ingredient. No organic solvents are used in the extraction of the Tongkat Ali Root Extract. Furthermore, the results of regular analyses for heavy metal and microbiological parameters support the absence of deleterious compounds originating from the production process (see Section I.C).

II.D Control of the Manufacturing Process

As previously mentioned, Tongkat Ali Root Extract is manufactured according to GMP, HACCP, and ISO 22000:2005 principles.

II.E Potential to Alter the Levels of Substances with an Adverse Effect on Public Health

There is no anticipated potential for Tongkat Ali Root Extract to alter the levels of substances with an adverse effect on public health.

II.F Potential Contamination of Micro-organisms of Adverse Health Significance

Microbiological contamination is minimised by several steps in the manufacturing process. Firstly, the raw material must meet microbiological standards prior to its use. Secondly, after the extraction and filtration process, the concentrate is subject to heat sterilisation. Finally, microbiological specifications have been established to ensure the absence of deleterious amounts of total bacteria, yeast and mould, salmonella, *Escherichia coli, Staphylococcus aureus*, can bile-tolerant gram-negative bacteria. The results of batch analyses and stability testing demonstrate the absence of such microbiological contaminants (see Sections I.C and I.E).

III HISTORY OF THE SOURCE ORGANISM – *Eurycoma longifolia*

Based on the SCF guidelines, the following questions must be addressed:

- "Is the novel food obtained from a biological source, *i.e.*, a plant, animal or microorganism?"
- "Has the organism used as the source of the novel food been derived using GM?"
- "Is the source organism characterised?"
- "Is there information to show that the source organism and/or foods obtained from it are not detrimental to human health?"

These questions have been addressed collectively in Section III.A and III.D.

III.A Biological Source

Biotropics' Tongkat Ali Root Extract ingredient is produced from the dried roots of *Eurycoma longifolia* (common name Tongkat ali), a slow-growing tree indigenous to Sumatra, Indonesia with pinnate, spiral leaves and panicle flowers (Elliott and Brimacombe, 1987; Bhat and Karim, 2010). This member from the Simaroubaceae family of plants is verified and authenticated with accompanying specimen vouchers from the Universiti Putra Malaysia.

III.B Derivation Using Genetic Modification

The raw materials and processing aids used in the production of Tongkat Ali Root Extract have not been genetically modified.

III.C Characterisation of the Source Organism

The taxonomic classification of the *E. longifolia* plant is presented below.

Kingdom:	Plantae
Order	Sapindales
Family	Simaroubaceae
Genus	Eurycoma
Species	longifolia

III.D Information to Support the Safety of the Source Organism and Foods Derived from It

Several publications describing the history of the consumption of *Eurycoma longifolia* as an herbal remedy are available (Oxley, 1850; Elliott and Brimacombe, 1987; Bhat and Karim, 2010; Ong *et al.*, 2012). Consumption of *E. longifolia* is prevalent in South-East Asian countries including Malaysia, Indonesia, and Vietnam. The *E. longifolia* plant has been used traditionally, with most of the parts of the plant having a history of consumption. In particular, the root extract of *E. longifolia* has been used by indigenous men to support healthy sexual appetite as well as general health and vitality. In women, the root extract has been reported to be used as an herbal remedy for restoring energy and vitality.

IV-VIII NOT APPLICABLE

IX INTAKE/EXTENT OF USE OF TONGKAT ALI ROOT EXTRACT

Based on the SCF guidelines, the following questions must be:

- “Is there information on the anticipated uses of the novel food based on its properties?”
- “Is there information to show anticipated intakes for groups predicted to be at risk?”
- “Will introduction of the novel food be restricted geographically?”
- “Will the novel food replace other foods in the diet?”

These questions have been addressed collectively in Sections IX.A through IX.D.

IX.A Intended Uses of Tongkat Ali Root Extract

Tongkat Ali Root Extract is proposed for use as an ingredient in beverages (coffee, sports and energy drinks, tea-based beverages), chocolate and confectionary (chocolate bars, candies), cereal bars, products for special nutritional use (nutrition and energy bars) and food supplements (including capsule form, tablet form, and liquid form). The proposed use level in these foods is from 50 to 75 mg per serving. The individual proposed food uses and use levels for Tongkat Ali Root Extract in conventional food matrices are presented in Table IX.A-1. All products containing Tongkat Ali Root Extract will be conspicuously labelled as intended for adult males and females and not for children under 18, pregnant, or lactating women.

Table IX.A-1 Summary of the Individual Proposed Food Uses and Use Levels for Tongkat Ali Root Extract in the European Union

FCS L2[a]	Food Category	Proposed Food-Uses	Serving Size[b] (g or mL)	Use Level (mg/serving)	Use Level (mg/100g)
A.01.06	Breakfast cereals	Cereal bars	40	50 to 75	125 to 187.5
A.10.03	Chocolate (Cocoa) products	Chocolate bars	40	50 to 75	125 to 187.5
A.10.04	Confectionery (non-chocolate)	Candies (for adults)[c]	40	50 to 75	125 to 187.5
A.13.02	Tea (Infusion)	Tea-based drinks	190	50 to 75	26.3 to 39.5
A.13.03	Coffee (beverage)	Coffee-based drinks	190	50 to 75	26.3 to 39.5
A.18	Products for special nutritional use (unspecified)	Nutrition bars	40	50 to 75	125 to 187.5
A.18.03	Food for sports people (labelled as such)	Energy bars	40	50 to 75	125 to 187.5
A.18.03	Food for sports people (labelled as such)	Sports and energy drinks	500	50 to 75	10 to 15

[a] Based on the Commission Regulation Food Classification System, Level 2.
[b] Serving sizes are based on the UK Food Portion Sizes Handbook (FSA, 2002).
[c] Tongkat Ali Root Extract is proposed for use in candies conspicuously labelled for adults; however, for the purposes of generating conservative estimates of intakes, it is noted that all candies were selected in the assessment below.

IX.B Anticipated Daily Intakes of Tongkat Ali Root Extract

In order to assess the potential consumption of Tongkat Ali Root Extract in the European Union, estimates were generated based on food consumption data from the European Food Safety Authority (EFSA) Comprehensive Database utilising the EFSA Food Additive Intake Model (FAIM) Tool (EFSA, 2013). This tool was used as a conservative method of estimating intakes of the ingredient from its proposed uses in conventional foodstuffs. A refined intakes assessment was conducted using the most recent data from the United Kingdom (UK) National Diet and Nutrition Survey (NDNS) rolling programme 2008-2012 (Department of Health, 2014; UKDA, 2014). Calculations for the mean and high-level

(95th percentile) all-person and all-user intakes, and percent consuming were performed for each of the individual proposed food-uses for Tongkat Ali Root Extract. Similar calculations were used to determine the estimated total intake of Tongkat Ali Root Extract from all proposed food-uses combined. Food products containing Tongkat Ali Root Extract are targeted toward male and female adults and will be conspicuously labelled as such. Toddlers and children are not anticipated to consume any products containing Tongkat Ali Root Extract as they are excluded population groups. Intakes in the teenager age group have been included below as a worst-case scenario in which this population may incidentally consume foodstuffs containing the ingredient.

A full description of the methodology used for the intake assessments and a detailed discussion of the results are provided in Appendix F of the confidential dossier.

IX.B.1 Intake Estimates Based on the EFSA Comprehensive Food Consumption Database and FAIM Tool

The intakes of Tongkat Ali Root Extract from its proposed uses were estimated using the EFSA FAIM tool (EFSA, 2013). The proposed uses of Tongkat Ali Root Extract (presented in Table IX.A.1 above) were matched as closely as possible with the Food Classification System Level 2 categories prescribed in the EFSA FAIM tool. In all cases, the maximum proposed use level was applied across the associated Food Classification System Level 2 category for the purposes of generating a conservative intakes estimate.

The results of the intakes assessment, expressed on a per kilogram body weight basis, are summarised in Table IX.B.1-1. Amongst adults, which are the target population group, the mean and heavy-level intakes of Tongkat Ali Root Extract from its proposed uses in conventional foods ranged from 0.8 to 5.9 mg/kg body weight/day and 3.3 to 12.1 mg/kg body weight/day, respectively. The heavy-level intakes are equivalent to intakes of 231 to 847 mg/person/day in a 70 kg individual (assuming 100% of all foods consumed contained the ingredient at the maximum proposed use level). Intakes in adolescents (aged 10 to 17 years) and the elderly (aged 65 years and older) were comparable to those reported in adults.

Table IX.B.1-1 Summary of Estimated Intakes of Tongkat Ali Root Extract from Proposed Food Uses Using the EFSA FAIM Tool

Population	Ages	Mean Intakes (mg/kg bw/day)		Heavy-level Intakes (mg/kg bw/day)	
		Minimum	Maximum	Minimum	Maximum
Adolescents	10 to 17 years	1.0	3.8	3.0	11.1
Adults	18 to 64 years	0.8	5.9	3.3	12.1
Elderly	65 years and older	1.0	5.9	2.4	11.2

Abbreviations: bw = body weight; EFSA = European Food Safety Authority; FAIM = Food Additives Intakes Model.

IX.B.1.1 Contribution of Individual Food Groups to Tongkat Ali Root Extract Intakes (EFSA FAIM)

For all population groups, Category 14.1.5 (representing the proposed uses of the ingredient in tea- and coffee-based drinks), was the main contributor to Tongkat Ali Root Extract intakes across all population groups, with minor contributions from energy bars and sport and energy drinks. Additional details are provided in Appendix F of the confidential dossier.

IX.B.2 Intake Estimate Based on the UK National Diet and Nutrition Survey (UK NDNS)

The NDNS is a programme of surveys designed to assess the diet, nutrient intake and nutritional status of people aged 1.5 years and older living in private households. The survey is carried out in all 4 countries of the UK and is designed to be representative of the UK population. The assessment used combined results from Years 1, 2, 3 and 4 of the Rolling Programme (2008 – 2012) for a sample of the UK population designed to be nationally representative.

For the intake assessment, mean and high percentile estimates for the intake of Tongkat Ali Root Extract by the UK population (teenagers, adults, and the elderly) were generated, using consumption data from individual dietary records, detailing food items ingested by each survey participant on each of the survey days, combined with the maximum proposed use levels for this ingredient as described in Table IX.A-1 above. Estimates for the daily intake of Tongkat Ali Root Extract represent projected 4-day averages for each individual from Days 1 to 4 of UK NDNS data. All-person intake refers to the estimated intake of Tongkat Ali Root Extract averaged over all individuals surveyed regardless of whether they consumed food products in which Tongkat Ali Root Extract is currently proposed for use, and therefore includes “zero” consumers (those who reported no intake of identified food products of interest during the 4 survey days). All-user intake refers to the estimated intake of this ingredient by those individuals consuming food products in which the use of Tongkat Ali Root Extract is currently under consideration, hence the ‘all-user’ designation. Individuals were considered users if they consumed 1 or more food products in which Tongkat Ali Root Extract is proposed for use on 1 of the 4 survey days.

Estimates for the total daily intakes of Tongkat Ali Root Extract from all proposed food-uses on an absolute basis and on a per body weight basis are provided in Tables IX.B.2-1 and IX.B.2-2, respectively. Adults represent the target population for which food products containing Tongkat Ali Root Extract would be marketed toward; however, intake data for teenagers (aged 11 to 18 years) are included below as a worst-case scenario in which this population group may incidentally consume foods containing this ingredient. Greater than 41.5% of all population groups consisted of users of those food products in which Tongkat Ali Root Extract is currently proposed for use.

The mean and 95th percentile all-user intakes of Tongkat Ali Root Extract among the target population of adults (male and female) were 56.4 and 165.9 mg/person/day, respectively. Male adults were determined to have the greatest mean and 95th percentile all-user intakes

of Tongkat Ali Root Extract on an absolute basis of 62.3 and 187.2 mg/person/day, respectively (Table IX.B.2-1). Teenagers and the elderly had comparable or lower mean and 95th percentile intakes to those of adults, even with the assumption that foods consumed by these non-target population groups would contain the ingredient at the maximum intended use level.

Table IX.B.2-1 Summary of the Estimated Daily Intake of Tongkat Ali Root Extract from All Proposed Food Categories in the UK by Population Group (NDNS Data, 2008-2012)

Population Group	Age Group (Years)	Total n	All-Person Consumption (mg/day)		All-Users Consumption (mg/day)			
			Mean	95th Percentile	%	n	Mean	95th Percentile
Teenagers	11-18	884	40.3	135.6	74.2	655	54.4	150.1
All adults	19-64	1,655	31.6	128.0	56.0	918	56.4	165.9
Female Adults	19-64	945	27.4	116.6	54.6	521	50.2	152.1
Male Adults	19-64	710	35.7	130.3	57.4	397	62.3	187.2
Elderly	≥65	428	19.9	77.4	41.5	176	47.9	161.7

Abbreviations: NDNS = National Diet and Nutrition Survey; UK = United Kingdom.

In the target population of adults, the mean and 95th percentile intakes among the user population were 0.75 and 2.26 mg/kg body weight/day, respectively (Table IX.B.2-2). With the worst-case scenario that foods consumed by teenagers would contain Tongkat Ali Root Extract at the maximum intended use level, the mean and 95th percentile intakes on a body weight basis were the highest in this population group at 0.99 and 3.01 mg/kg body weight/day, respectively. However, it is important to note that teenagers are not the intended population group and the intake assessment model assumes all foods in which Tongkat Ali Root Extract is proposed for use contain the ingredient at the maximum intended use level. Thus, this data is presented for informational purposes and is not intended to model real-world exposures.

Table IX.B.2-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Tongkat Ali Root Extract from All Proposed Food Categories in the UK by Population Group (NDNS Data, 2008-2012)

Population Group	Age Group (Years)	Total n	All-Person Consumption (mg/kg bw/day)		All-Users Consumption (mg/kg bw/day)			
			Mean	95th Percentile	%	n	Mean	95th Percentile
Teenagers	11-18	853	0.74	2.72	74.1	631	0.99	3.01
All adults	19-64	1,545	0.43	1.71	57.1	873	0.75	2.26
Female Adults	19-64	878	0.41	1.67	55.7	493	0.73	2.34
Male Adults	19-64	667	0.45	1.76	58.5	380	0.77	2.21
Elderly	≥65	393	0.28	1.21	42.4	165	0.65	2.09

Abbreviations: bw = body weight; NDNS = National Diet and Nutrition Survey; UK = United Kingdom.

IX.B.2.1 Contribution of Individual Food Groups to Tongkat Ali Root Extract Intakes (UK NDNS)

The total adult UK population was identified as being significant consumers of chocolate bars (38.0%) and coffee-based drinks (11.7%). The remaining individual food categories were generally consumed by less than 11.5% of the adult population.

IX.B.3 Food Supplements

Tongkat Ali Root Extract also is intended to be an ingredient in food supplements marketed to adult men and women. The proposed use level of Tongkat Ali Root Extract is up to 200 mg/day, equivalent to approximately 2.86 mg/kg body weight/day for a 70 kg adult. Food supplements containing Tongkat Ali Root Extract would be consumed as an alternative source of Tongkat Ali Root Extract in conventional food forms and will be conspicuously labelled as such; therefore, it is not expected nor recommended that individuals will consume both supplements and foods containing Tongkat Ali Root Extract.

IX.B.4 Conclusions Regarding Intakes of Tongkat Ali Root Extract

The intakes of Tongkat Ali Root Extract from its proposed uses were estimated using the EFSA FAIM tool and consumption data from the UK NDNS. Amongst adults, which are the target population group, the mean and heavy-level intakes of Tongkat Ali Root Extract from its proposed uses in conventional foods ranged from 0.8 to 5.9 mg/kg body weight/day and 3.3 to 12.1 mg/kg body weight/day, respectively, using the EFSA FAIM tool. Among the non-target adolescent population, the estimated mean and heavy-level intakes were lower and ranged from 1.0 to 3.8 mg/kg body weight/day and from 3.0 to 11.1 mg/kg body weight/day, respectively.

A more refined intake assessment was conducted using data from the UK NDNS. The all-user mean and 95th percentile calculations for the target population (*i.e.*, adults) resulted in intake estimates of 56.4 and 165.9 mg/day, respectively (equivalent to 0.75 and 2.26 mg/kg body weight/day). The results of the assessment indicate that the highest worst-case 95th percentile Tongkat Ali Root Extract intakes for the non-target teenager population group was up to 150.1 mg/day (equivalent to 3.01 mg/kg body weight/day on a per kilogram body weight basis).

Tongkat Ali Root Extract also is intended to be an ingredient in food supplements marketed to adult men and women. The proposed use level in food supplement forms is 200 mg/day. Food supplements containing Tongkat Ali Root Extract would be consumed as an alternative source of Tongkat Ali Root Extract in conventional food forms and will be conspicuously labelled as such; therefore, it is not expected that individuals will consume both supplements and foods containing Tongkat Ali Root Extract.

IX.C Geographical Restrictions

The marketing of food and food supplements containing Tongkat Ali Root Extract will not be restricted geographically.

IX.D Will the Novel Food Replace Other Foods

Tongkat Ali Root Extract is not intended to replace other foods currently on the market.

X INFORMATION FROM PREVIOUS HUMAN EXPOSURE TO THE NOVEL FOOD OR ITS SOURCE

Based on the SCF guidelines, the following question must be answered:

- Is there information from previous direct, indirect, intended or unintended human exposure to the novel food or its source which is relevant to the Community situation with respect to production, preparation, population, lifestyles and intakes?

This question is addressed in Section X.A below.

X.A Information from Previous Human Exposure

Although there is only limited evidence of consumption of the ingredient in the Community (dating back approximately 15 years), Biotropics Malaysia Berhad's Tongkat Ali Root Extract has been distributed in various international markets. A summary of the regions in which products containing Tongkat Ali Root Extract are marketed is summarised in Table X.A-1.

Table X.A-1 Countries in Which Products Containing Tongkat Ali Root Extract are Currently Marketed

Country/Region	Product Category	Year of Market Entry
Canada	Natural health product	2012
United States	Dietary supplement	2013
Japan	Herbal food ingredient	2011
Singapore	Traditional medicine	2009
Russia	Food supplement/Biological active food supplement	2011/2012
Hong Kong	Herbal-based product (traditional)	2010
Malaysia	Traditional medicine	2008

To date, there have not been reports of adverse effects following consumption of Biotropics Malaysia Berhad's Tongkat Ali Root Extract ingredient. The total global circulated volume for Tongkat Ali Root Extract in 2014 was approximately 3,015.66 kg (equivalent to 15.0 million servings of 200 mg or 60.31 million servings of 50 mg).

XI NUTRITIONAL INFORMATION ON TONGKAT ALI ROOT EXTRACT

Based on the SCF guidelines, the following question must be answered:

- “Is there information to show that the novel food is nutritionally equivalent to existing foods that it might replace in the diet?”

This question has been addressed in Section XI.A and XI.B.

XI.A Nutritional Equivalence to Existing Foods

Tongkat Ali Root Extract is not nutritionally equivalent to other foods and is not intended to replace other foods currently on the market in the EU.

XI.B Other Nutritional Considerations

A summary of the proximate analysis and elemental composition of Tongkat Ali Root Extract is presented in Table XI.B-1. Based on the results, Tongkat Ali Root Extract is not anticipated to impact the quality of the diet nor play any role in the diet. Tongkat Ali Root Extract is not anticipated to modulate the nutritional properties of the foods to which it is intended to be added.

Table XI.B-1 Proximate Analysis and Elemental Composition of Tongkat Ali Root Extract

Parameter	Concentration in Tongkat Ali Root Extract	Anticipated Intake per 200 mg Serving	Dietary Reference Values[1]
Proximate Analysis			
Energy (kcal/100g)	~360 to 380	Up to 0.76 kcal	AR 1,614 to 3,340 kcal/day
Fat (g/100g)	<0.20	<0.4 mg	20 to 35% total energy
Carbohydrate (g/100g)	~80 to 92	184 mg	45 to 60% total energy
Total sugar (g/100g)	~13	26 g	Were not established
Protein (g/100g)	~4 to 10	Up to 20 mg	AR 0.66 g/kg bw/day
Elemental Composition			
Sodium	770 mg/kg	0.154 mg	WHO < 2 g/day
Calcium	1,964 mg/kg	0.39 mg	AR 750 mg/day
Chromium	0.14 mg/kg	<0.001 mg	Were not established
Iron	10.60 mg/kg	0.002 mg	AR 6 mg/day
Magnesium	2,206 mg/kg	0.441 mg	AI 300 to 350 mg/day
Manganese	142 mg/kg	0.028 mg	AI 3 mg/day
Phosphorus	249 mg/kg	0.050 mg	AI of 550 mg/day
Potassium	1,511 mg/kg	0.302 mg	WHO 3,510 mg/day
Zinc	3.38 mg/kg	<0.001 mg	AR 6.2 to 12.7 mg/day
Vitamins			
Vitamin B2	0.39 mg/100 g	<0.001 mg	Not available
Vitamin B3	0.46 mg/100 g	<0.001 mg	Not available
Vitamin B5	0..36 mg/100 g	<0.001 mg	Not available
Vitamin B6	0.06 mg/100 g	<0.001 mg	Not available
Vitamin B12	1.9 µg/100 g	<0.001 µg	AI 4 µg/day
Folic acid	99.2 µg/100 g	0.02 µg	AR 250 µg dietary folate equivalents
Vitamin E	0.11 mg/100 g	<0.001 mg	AI 11 to 13 mg/day

Abbreviations: AI = adequate intake; AR = average requirement; bw = body weight; ND = not detected; WHO = World Health Organisation.
Note: No appreciable levels of aluminium, cobalt, copper, molybdenum, nickel, selenium, vitamin A, vitamin C, or vitamin D3 were detected.
[1] As established by EFSA unless otherwise specified. See http://www.efsa.europa.eu/en/topics/topic/drv

XII MICROBIOLOGICAL INFORMATION ON TONGKAT ALI ROOT EXTRACT

Based on the SCF guidelines, the following question must be addressed:

- “Is the presence of any microorganisms or their metabolites due to the novelty of the product/process?”

This question has been addressed in Section XII.A.

XII.A Microbiological Specifications and Analysis for Tongkat Ali Root Extract

The microbiological specifications and batch analyses were presented in Table I.C-1 and reproduced in Table XII.A-1 below. The results confirm that the production process does not introduce a potential for microbiological contamination and the final Tongkat Ali Root Extract ingredient is free from microbial contaminants, even after 36 months of storage (see Table I.E-1).

Table XII.A-1 Microbiological Specifications and Batch Analyses for Tongkat Ali Root Extract

Parameter	Specifications	TA 130511	TA 130831	TA 130935
Total bacteria count (CFU/g)	< 10,000	< 10	< 10	< 10
Yeast and mould (CFU/g)	< 100	< 10	< 10	< 10
Salmonella (/10g)	Absent	Absent	Absent	Absent
Escherichia coli (/1g)	Absent	Absent	Absent	Absent
Staphylococcus aureus (/1g)	Absent	Absent	Absent	Absent
Bile-tolerant gram-negative bacteria (bacteria/g)	< 100	< 10	< 10	< 10

Abbreviations: CFU = colony forming units.

XIII TOXICOLOGICAL INFORMATION ON TONGKAT ALI ROOT EXTRACT

Based on the SCF guidelines, the following questions must be addressed:

- “Is there a traditional counterpart to the novel food that can be used as a baseline to facilitate the toxicological assessment?”

- “Compared to the traditional counterpart, does the novel food contain any new toxicants or changed levels of existing toxicants?”

or

- “Is there information from a range of toxicological studies appropriate to the novel food to show that the novel food is safe under anticipated conditions of preparation and use?”

- “Is there information which suggests that the novel food might pose an allergenic risk to humans?”

These questions have been addressed collectively in Sections XIII.A to XIII.E.

XIII.A Absorption, Distribution, Metabolism, and Elimination (ADME)

The bioavailability and pharmacokinetics of the characteristic component of Tongkat Ali Root Extract, eurycomanone, have been evaluated in rats using a validated high-performance liquid chromatography (HPLC) assay (Low *et al.*, 2005). An isolated and purified preparation of eurycomanone from the roots of *E. longifolia* (57.8%) was obtained by 50% aqueous ethanol extraction and singly administered to male Sprague-Dawley rats (5/group) by intravenous injection (in saline) at a dose of 1.96 mg/kg body weight. Blood samples were obtained from the rats at baseline (pre-dose), 20 minutes, 40 minutes, and at 1, 2, 4, 6, and 8 hours post-dose. Following a 2-week washout period, the same rats were administered the eurycomanone preparation by feeding needle at 9.8 mg/kg body weight and blood samples were obtained at baseline, 1, 2, 4, 6, 8, 10, 12, and 16 hours post-administration. The plasma was analysed using a validated HPLC method with an accuracy range within the acceptable 80 to 100% range for bioanalytical method validation.

The pharmacokinetic values that were determined following intravenous and oral administration are summarised in Table XIII.A-1.

Table XIII.A-1 Pharmacokinetic Values for the Bioactive Component of Tongkat Ali Root Extract, Eurycomanone (Low *et al.*, 2005)

Parameter (Units)	Oral administration	Intravenous administration
Area under the plasma concentration-time curve, $AUC_{0 \rightarrow \infty}$ (µg h/mL)	3.11 ±0.35	5.93 ± 1.24
Peak concentration, C_{max} (µg/mL)	0.33 ± 0.03	-
Time to peak concentration, T_{max} (h)	4.40 ± 0.98	-
Half-life, $T_{1/2}$ (h)	-	1.00 ± 0.26
Volume of distribution, V_d (L/kg)	-	0.68 ± 0.30
Elimination rate constant, K_e (h^{-1})	-	0.88 ± 0.19
Clearance, CL (L/h/kg)	-	0.39 ± 0.08

Abbreviations: - = not available. Values are presented as mean ± standard error.

The low bioavailability of the eurycomanone (10.5%) following oral administration is anticipated to be due to pre-systemic metabolism or the first-pass effect prior to reaching the systemic circulation. Eurycomanone also exhibits a short half-life in the systemic circulation as evidenced by the half-life of approximately 1 hour following intravenous administration.

XIII.B Toxicological Studies

XIII.B.1 Acute Toxicity Studies

The acute oral toxicity of Tongkat Ali Root Extract was evaluated in Wistar rats according to Organisation for Economic Co-operation and Development (OECD) Test Guideline 420 (Choudhary *et al.*, 2012). The oral median lethal dose (LD_{50}) of Tongkat Ali Root Extract was determined to be greater than 2,000 mg/kg body weight. Therefore, Tongkat Ali Root Extract is not considered to be an acute oral toxicant.

XIII.B.2 Repeated Dose Toxicity Studies

XIII.B.2.1 28-Day Repeat Dose Oral Toxicity Study

A sub-acute repeat dose oral toxicity study was conducted with Tongkat Ali Root Extract in accordance with OECD Guideline 407 (Choudhary *et al.*, 2012). Wistar rats (5/sex/group) were administered Tongkat Ali Root Extract at doses of 0 (water control), 250, 500, or 1,000 mg/kg body weight/day for 28 days by oral gavage. Additional groups receiving 0 (control) and 1,000 mg/kg body weight/day were sacrificed following a 14-day recovery period. All rats were monitored for body weight, food intake and clinical signs of toxicity. Blood samples were obtained at baseline and at the end of the treatment period and analysed for haematological (including red blood cell count, haemoglobin, haematocrit, white blood cells, platelets, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, and mean corpuscular volume) and biochemical parameters (including total protein, albumin, glucose, aspartate aminotransferase, alanine aminotransferase, urea creatinine, cholesterol, total bilirubin, sodium, and potassium). Ophthalmological examination, sensory evaluations, and motor activity (as evaluated using an animal activity meter) were recorded at baseline and during the fourth week of treatment, and on the second week of the recovery period. At the end of the study, rats were subject to necropsy, which included macroscopic and histopathological examination and organ weight).

No mortalities or clinical signs of toxicity were observed during the treatment period. No toxicologically significant changes in body weight gain, feed consumption, haematological parameters, or blood biochemical parameters was observed compared to controls. No differences in sensory, motor, or grip strength were reported between groups, and no abnormalities were reported during ophthalmological examination. No test article-related differences in organ weights or any histopathological findings were reported.

Based on the results of the study, a no-observed-adverse-effect level (NOAEL) of 1,000 mg/kg body weight/day, the highest dose tested, was established for both male and female rats.

XIII.B.2.2 90-Day Repeat Dose Oral Toxicity Study

A sub-chronic repeat dose oral toxicity study was conducted with Tongkat Ali Root Extract in accordance with OECD Guideline 408 (Choudhary *et al.*, 2012). Wistar rats (10/sex/group) were administered Tongkat Ali Root Extract at doses of 0 (water control), 250, 500, or 1,000 mg/kg body weight/day for 90 days by oral gavage. Additional groups receiving 0 (control) and 1,000 mg/kg body weight/day were sacrificed following a 28-day recovery period. Animals were monitored for body weight, food consumption, and clinical signs of toxicity. Opthalmoscopic examination was conducted at baseline and at the end of the treatment period. Detailed clinical assessments (including changes in gait, posture, response to handling, clonic or tonic movements, stereotypes and bizarre behaviour) also were conducted during treatment and recovery. On Days 86 or 87, rats underwent a functional observational battery (FOB). Blood samples were collected at baseline and at the

end of the treatment and recovery periods and analysed for haematological (including red blood cells, white blood cells, haemoglobin, packed cell volume, platelets, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, mean corpuscular volume, differential white blood cell count) and blood biochemical parameters (including total protein, albumin, globulin, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, glucose, urea nitrogen, urea, creatinine, total bilirubin, calcium, phosphorus, total cholesterol, triglycerides, sodium, and potassium). Urine samples also were collected at the end of the treatment and recovery periods and analysed microscopically for the presence of epithelial cells, leucocytes, erythrocytes, casts, granular casts, crystals, other abnormal constituents, and triple phosphate crystals. Rats were sacrificed and underwent necropsy (including organ weight, macroscopic, and histopathological examination).

No test article-related mortality or clinical signs of toxicity were noted in any animals during the treatment period. No significant differences in body weight gain, feed consumption, sensory parameters, motor parameters, grip strength, haematological and blood biochemical parameters, urinalysis parameters, organ weights, or histopathological observations were observed in treated animals compared to controls.

The NOAEL of Tongkat Ali Root Extract was concluded to be 1,000 mg/kg body weight/day, the highest dose tested, for both male and female rats.

XIII.B.2.3 12-Month Repeat Dose Oral Toxicity Study

A chronic repeat dose oral toxicity study was conducted with Tongkat Ali Root Extract in accordance with OECD Guideline 452 (Gohel, 2015 [unpublished]). Wistar rats (25/sex/group) were administered Tongkat Ali Root Extract at doses of 0 (water control), 250, 500, or 1,000 mg/kg body weight/day for 12 months by oral gavage. Satellite groups (12/sex/group) using the identical doses were included for interim sacrifice. Additional groups (25/sex/group) receiving 0 (control) or 1,000 mg/kg body weight/day were sacrificed following a 28-day recovery period. A negative control group (5/sex) also was included. The animals were monitored daily for mortality, morbidity, and clinical signs of toxicity. Body temperature, body weight and food consumption were measured weekly. At the end of Week 1 and then at monthly intervals thereafter, a detailed clinical and neurobehavioural observation (posture, convulsions, ease of removing rat from cage, handling reactivity, palpebral closure, lacrimation, eye examination, piloerection, skin examination, salvation, gait, mobility score, arousal level, vocalizations, rearing, respiration, clonic or tonic movements, urination and defecation, stereotypy, bizarre behaviour) was conducted. FOB was conducted at 0, 3, 6, 9, and 12 months and Week 4 of recovery period, and complete ophthalmological examination was conducted at 0, 6, and 12 months and Week 4 of recovery period. Blood samples were collected prior to treatment, at Week 2, at 3, 6, 9, 12 months, and Week 4 of recovery period for haematological (including white blood cell, erythrocyte, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, platelets, reticulocyte, differential leukocyte, prothrombin time, activated partial thromboplastin time) and blood

biochemical parameters (including alanine aminotransferase, albumin, aspartate aminotransferase, alkaline phosphatase, blood urea nitrogen, calcium, creatinine, creatine kinase, gamma glutamyl transpeptidase, glucose, inorganic phosphorus, total bilirubin, total protein, triglyceride, total cholesterol, urea, potassium, sodium, chloride, globulin, albumin:globulin ratio). Urine samples were collected prior to treatment and at 3, 6, 9, and 12 months for 10/sex/group, and at end of treatment and recovery periods for all animals, for physical observation (including appearance, colour, volume, and microscopic evaluation of sediment) and chemical observation (including specific gravity, pH, protein, glucose, ketones, blood, bilirubin, urobilinogen). Rats were killed, underwent gross examination, and all organs were removed, examined, weighed, and prepared for histological examination.

No mortality or morbidity was observed in females, and no treatment related mortality or morbidity was observed in males. No toxicological relevant or treatment related clinical signs of toxicity, ophthalmological abnormalities, FOB observations, or clinical and neurobehavioural observations were reported. Minor changes in haematological, clinical chemistry, urinalysis, and organ weight parameters were observed. Significant increases in platelet, white blood cell, neutrophil, lymphocyte, potassium, phosphorus, creatinine kinase, pH of urine, absolute and relative weights of livers, kidneys, posterior prostate, thyroid, parathyroid, and spleen, and relative weight of heart was observed in all treated groups. Significant decreases in mean corpuscular volume, mean corpuscular haemoglobin, haemoglobin, blood urea nitrogen/urea, cholesterol, chloride, sodium, terminal body weight, alkaline phosphatase (males only), albumin/total protein, and creatinine (females only) was observed. The majority of these effects occurred in a dose-dependent manner, with the low-dose group included in some cases, and almost all effects became non-significant during the recovery period. These effects were small in magnitude of effect, lacked consistency between males and females, may have been related to increased muscular activity, commonly observed in historical data, related to antigenicity of test article, and/or related to terminal body weight changes. None of these effects were in association with histological lesions and as such, the authors considered these effects as adaptive responses to the compound and not as adverse.

Based on the results of this study, the NOAEL for Tongkat Ali Root Extract was determined to be 1,000 mg/kg body weight/day, the highest dose tested, for male and females.

XIII.B.3 Developmental and Reproductive Toxicity

XIII.B.3.1 Reproductive and Developmental Toxicity Screening Test

The reproductive and developmental toxicity of Tongkat Ali Root Extract was evaluated in a reproductive and developmental toxicity screening study conducted in accordance with OECD Testing methods 421 (Takawale, 2011 [unpublished]). Wistar rats (10/sex/group) were administered Tongkat Ali Root Extract at doses of 0 (control), 250, 500, or 1,000 mg/kg body weight/day for 14 days prior to mating, followed by cohabitation for up to 14 days. Males were sacrificed on Day 29 of treatment (including the 14 day pre-mating period), and

females (receiving the test article throughout the study period) were sacrificed on Post-Natal Day 4.

Body weight and feed consumption was recorded for male and female rats during the treatment period. Number of live litters, mean litter weight, total litter weight, male litter weight, and female litter weight were recorded on Post-Natal Days 0 and 4. Reproductive performance (precoital interval, duration of gestation, number of corpora lutea, number of implantation sites, proportion of pre-implantation loss, number of pups per litter, number of live pups on Post-Natal Days 0 and 4) was recorded. Pups were examined for gross external abnormalities. Dams underwent necropsy and histopathological evaluation (including number of corpora lutea, number of implantation sites, percent pre-implantation loss, and post-implantation loss).

No mortalities occurred during the treatment period. Clinical signs noted in rats receiving 500 and 1,000 mg/kg body weight/day included the following: piloerection, aggressive behaviour, moving the beddings, salivation, and nasal discharge (statistical significance not reported). The clinical signs of salivation and moving the bedding were observed immediately after administration and for a "short period". As well, salivation is a known clinical sign that may be related to the procedure of oral gavage. As such, the authors considered these clinical signs to be related to discomfort due to a local reaction to the test item and not due to a systemic effect. The clinical sign of aggressive behaviour was observed in control animals as well as treated animals. Furthermore, none of the clinical signs (*i.e.*, piloerection, aggressive behaviour, moving the beddings, salivation, and nasal discharge) occurred consistently nor on continuous days, and were sporadic and only observed on a limited number of occasions. Moreover, no adverse effects on overall health were observed in the study. These clinical signs were, therefore, not considered toxicological significant.

A transient but statistically significant decrease in body weight gain was observed in high-dose males between Day 28 and 29. In high-dose females, a decrease in body weight gain was observed during pre-mating Days 1 through 7, but not on pre-mating Days 7 through 14. A statistically significant decrease in body weight gain also was observed in dams receiving 250 mg/kg body weight (but not at higher doses). Taken together, it was concluded that no dose relationship could be established, effects were transient, and thus, these observed differences in body weight were considered toxicologically not relevant. A significant increase in food consumption was observed in males and females receiving 500 and 1,000 mg/kg body weight/day during pre-mating Days 7 through 14, which was correlated with the changes in body weight during this period. A significant increase in food consumption also was observed in females receiving 250 mg/kg body weight/day during Day 0 to 7 of gestation, and a decrease (not statistically significant) in food consumption was observed in females receiving 1,000 mg/kg body weight/day from Gestational Day 7 to Lactation Day 4. These changes also were correlated with changes in body weights during this time period. Due to a lack of consistent effect and no dose relationship, no toxicological relevance can be attributed to these findings.

No remarkable findings were noted upon necropsy and gross and/or histopathological examination of the males, females, and pups.

Based on the results of the study, the NOAEL for reproductive and developmental toxicity of Tongkat Ali Root extract was determined to be 1,000 mg/kg body weight/day, the highest dose tested.

XIII.B.3.2 Effects on Male Reproductive System

The effect of Biotropics Malaysia Berhad's Tongkat Ali Root Extract on sperm has been evaluated in Sprague-Dawley rats. Male rats (14/group) were administered Tongkat Ali Root Extract at doses of 0 (control), 200, or 800 mg/kg body weight/day for 14 days by oral gavage (Solomon *et al.*, 2014). Animals were monitored for overt signs of toxicity and body weights were recorded during the treatment period. At the end of the treatment period, blood samples were obtained to determine serum testosterone, and rats were euthanised and the testes, epididymis, seminal vesicles, and prostate gland were weighed. Spermatozoa were collected from the cauda epididymis and sperm count, sperm motility, sperm vitality, acrosomal status (a marker of sperm function), and sperm mitochondrial membrane potential (a marker of sperm motility) were assessed.

No overt signs of toxicity were observed during the treatment period. An increase in serum testosterone was observed rats provided 800 mg/kg body weight/day (+30.2%; from 0.86 ng/mL in controls to 1.12 ng/mL in high-dose rats); however, the increase was determined to be not statistically significant following ANOVA trend analysis. Values remained within high normal ranges for this strain of rats. Statistically significant decreases in body weight and omentum fat[1] weight were observed in animals receiving 800 mg/kg body weight/day compared to controls. No significant differences in the absolute organ weights of the male reproductive organs were observed in rats administered Tongkat Ali Root Extract compared to the controls. With respect to semen parameters, there were significant improvement on sperm concentration, motility and vitality, but no significant changes in mitochondrial membrane potential (MMP) nor acrosomal status; these changes show no deleterious effect to sperm function. The decreases in body weight and omentum fat were noted by the study investigators to be possibly secondary to the increase in serum testosterone concentration. Taken together, there were no adverse effects noted following administration of the Tongkat Ali Root Extract at doses of up to 800 mg/kg body weight/day (equivalent to 56 g/day in a 70 kg individual).

XIII.B.4 Mutagenicity and Genotoxicity

XIII.B.4.1 In vitro Assessments of Genotoxicity

A bacterial reverse mutation assay conducted in accordance with OECD Testing Guideline 471 was undertaken to evaluate the genotoxic potential of Tongkat Ali Root Extract (Ming *et al.*, 2014). In the experiment, *Salmonella typhimurium* strains TA98, TA100, TA102,

[1] Omentum fat was measured from the greater curvature of the stomach.

TA1535, and TA1537 were exposed to Tongkat Ali Root Extract using the standard plate incorporation method at concentrations of 0.005, 0.01, 0.03, 0.05, 0.3, 1.0, 3.0, or 5.0 mg/plate in the presence and absence of metabolic activation (S9 mix).

No significant differences in the numbers of revertant colonies were observed at any concentration compared to the negative controls. Based on the results, it was concluded that Tongkat Ali Root Extract was not mutagenic under the conditions of the assay.

The genotoxic potential of Tongkat Ali Root Extract was further evaluated in an *in vitro* mammalian cell gene mutation test in L5178Y mouse lymphoma cells (Verbaan, 2013 [unpublished]). The study was conducted in accordance with OECD Testing Guideline 476. L5178Y mouse lymphoma cells were exposed to Tongkat Ali Root Extract at concentrations of 800, 1,000, or 3,000 µg/mL both in the presence or absence of metabolic activation (S9 mix). It was noted that precipitation was observed at concentrations of 1,250 µg/mL and upwards.

In the absence of metabolic activation (S9), Tongkat Ali Root Extract induced a 2.6- to 4.5-fold statistically significant increase in mutation frequency at a concentration of 3,000 µg/mL. However, no statistically significant increases in mutation frequency were observed at 800 and 1,000 µg/mL. Similarly, no increases in mutation frequency were observed in the presence of metabolic activation. Taken together, the increase in mutation frequency was noted to occur only at severely toxic and precipitating dose levels, and thus, the increases were considered "not biologically relevant" by the study authors. It is well-recognised that high and precipitating testing concentrations may produce false positive responses (EFSA, 2012) and therefore, Tongkat Ali Root Extract is considered to be not mutagenic under the conditions of the assay. However, a follow-up *in vivo* assessment of genotoxicity was undertaken as per EFSA Guidelines (see following section).

XIII.B.4.2 In vivo Assessments of Genotoxicity

A mouse erythrocyte micronucleus assay was conducted with Tongkat Ali Root Extract in accordance with OECD Testing Guideline 474 (Ming *et al.*, 2014). NMRI mice (5/sex/group) were administered a single dose of 0 (negative control), 100, 250, or 500 mg/kg body weight by intraperitoneal injection. An additional group received 40 mg/kg body weight of cyclophosphamide as a positive control. Animals were sacrificed 24 hours post-administration and erythrocytes were collected for analysis.

No significant differences in the ratios of polychromatic erythrocytes to normochromatic erythrocytes were observed between groups. No increases in the frequency of micronucleated polychromatic erythrocytes were observed compared to the negative control, whereas the positive control produced the expected response. Therefore, the results of the study suggest that Tongkat Ali Root Extract is not genotoxic.

XIII.B.5 Carcinogenicity

Studies evaluating the carcinogenic potential of Tongkat Ali Root Extract are not available.

XIII.C Human Studies

XIII.C.1 Safety Studies

The effects of oral administration of Tongkat Ali Root Extract on supporting men's health have been evaluated in several studies; however, the studies summarised in this section include only those in which the safety of Biotropics' Tongkat Ali Root Extract (meeting the standardisation criteria described in this dossier) is investigated.

In a randomised, double-blind, placebo-controlled, parallel designed study conducted in accordance with Good Clinical Practice, 109 healthy men or those with stable chronic medical illnesses[2] (ages 30 to 55 years) were provided capsules containing 75 mg of Tongkat Ali Root Extract (31.75% total protein, 41.08% glycosaponin, and 1.604% eurycomonone) or placebo, to be taken 4 times a day (for a total dose of 300 mg/day or placebo) for 12 weeks (Ismail *et al.*, 2012). Safety parameters included a quality of life questionnaire, adverse event monitoring, physical examination, clinical and laboratory measures (including blood urea serum electrolytes, creatinine, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatise, total protein, albumin, globulin, bilirubin, prostate-specific antigen, glucose, uric acid, lipid profile, full blood count, and testosterone-to-epitestosterone ratio). Hormonal profiles (including free testosterone, serum total testosterone, Insulin-like Growth Factor 1 (IGF-1), dehydroepiandrosterone sulphate, sex hormone binding globulin) also were recorded, as well as efficacy parameters related to sexual function and physical fitness.

At the end of the study, no adverse effects on quality of life parameters compared to placebo were observed. Although there were statistically significant changes in laboratory parameters (uric acid, serum creatinine, and potassium), these were observed in both the Tongkat Ali Root Extract and the placebo groups and thus were deemed to be not clinically significant by the study investigators. One serious adverse event occurred during the study (low back pain requiring hospitalisation) in a subject receiving Tongkat Ali Root Extract; however, this was considered to be not related to treatment. A total of 31 adverse events in 26 subjects were reported; all of which were deemed to be "unlikely" related to the test article with the exception of a headache event in a subject receiving placebo (deemed to be "probable"). The study authors concluded that the daily dose of 300 mg Tongkat Ali Root Extract for 3 months was well-tolerated and safe compared to placebo.

In a comparative study, 13 physically active senior men (mean age of 65.9 years) and 12 physically active senior women (mean age of 63.1 years) were provided capsules containing 200 mg Tongkat Ali Root Extract (24.8% total protein, 44.24% glycosaponine, and 1.06% eurycomanone), to be taken twice daily for 5 weeks (for a total dose of 400 mg/day of Tongkat Ali Root Extract) (Henkel *et al.*, 2014). Haematological parameters (including haematocrit, haemoglobin, red blood cell count, white blood cell count, platelets, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, red cell distribution

[2] These included subjects with controlled diabetes mellitus and/or hypertension on mono-therapy or low dose combination therapy.

width, microcorpuscular volume), blood hormone levels (including total and free testosterone, dihydroepiandrosterone, cortisol, insulin-like growth factor-1, and sex hormone-binding globulin), blood biochemical parameters (including blood urea nitrogen, creatinine kinase) and a handgrip test (as a measure of muscle strength) were assessed at baseline, 3 weeks, and 5 weeks of treatment.

At 5 weeks, a statistically significant decrease in creatinine kinase and statistically significant increases in haemoglobin concentration, blood urea nitrogen, total testosterone, and free testosterone were observed in male subjects receiving Tongkat Ali Root Extract compared to baseline. With regards to the increase in blood urea nitrogen levels, the authors noted that the values were maintained within normal ranges. The remaining changes in laboratory parameters were regarded to be beneficial. Total testosterone increased from 3.84 ng/mL at baseline to 4.42 ng/mL at 5 weeks and free testosterone increased from 5.20 pg/mL at baseline to 8.38 pg/mL at 5 weeks. All testosterone concentrations remained within normal ranges[3]. A statistically significant increase in handgrip test also was observed at 5 weeks in men and women receiving Tongkat Ali Root Extract compared to baseline.

In females, statistically significant decreases in platelets and sex hormone binding protein, and significant increases in mean corpuscular haemoglobin concentration, total testosterone, and free testosterone were observed in females receiving Tongkat Ali Root Extract compared to baseline. The increases in testosterone in female participants were noted to remain within normal reference ranges and were not considered a concern. No further comment was provided regarding the remaining differences in blood parameters in females.

This study was not placebo-controlled, and thus, the effect of Tongkat Ali Root Extract itself is difficult to ascertain. However, the authors concluded that Tongkat Ali Root Extract "had no adverse effects and is acceptable to the senior recreational athlete as a form of health supplement for general well-being."

XIII.C.2 Other Studies

In addition to the pivotal safety studies summarised above, Tongkat Ali Root Extract has been evaluated in a number of other published studies primarily examining efficacy endpoints. Although these studies do not specifically include the results of safety parameters, they lend further support that no adverse effects are anticipated from the consumption of Tongkat Ali Root Extract at doses of 100, 200, or 300 mg/day for up to 12 weeks (see Table XIII.C.2-1).

[3] Normal ranges for total and free testosterone in males are 2.4 to 9.5 ng/mL and 90 to 300 pg/mL (in males 19 years and older), respectively.

Table XIII.C.2-1	**Other Clinical Studies Conducted on Tongkat Ali Root Extract**			
Study Population	**Dose and Duration of Tongkat Ali Root Extract Treatment**	**Parameters Examined (as reported)**	**Relevant Findings Regarding Safety**	**Reference**
14 healthy men	Single dose of 100 mg Tongkat Ali Root Extract or placebo	• Fat free mass • Muscle strength • Muscle mass	None.	Hamzah and Yusof, 2003 (also described in Tambi, 2009)
30 men	Single dose of 100 mg Tongkat Ali Root Extract or placebo	• Saliva cortisol and testosterone levels during intense endurance exercise (24-hour mountain biking event)	Saliva testosterone levels were increased by 16.4% compared to placebo after intense exercise.	Talbott *et al.*, 2006
76 men with late-onset hypogonadism (mean age 51 years)	200 mg/day Tongkat Ali Root Extract or placebo for 1 month	• Ageing Males' Symptoms Rating Scale • Serum testosterone	Serum testosterone was increased by 46.8% compared to baseline in this subject population having low testosterone levels; the proportion of subjects with testosterone levels within normal ranges improved from 35.5% at baseline to 90.8% following treatment with Tongkat Ali Root Extract.	Tambi *et al.,* 2012
32 men and 31 women with "moderate stress levels"	200 mg/day Tongkat Ali Root Extract or placebo for 4 weeks	• Mood state and hormone profile. • Liver enzyme markers (alanine aminotransferase and aspartate aminotransferase) • Body weight • Body Fat	Two subjects receiving Tongkat Ali Root Extract and 1 subject receiving placebo reported feeling "unusually fatigued" during the first 2 weeks of the study. No other adverse events were reported. No significant changes in markers of liver function markers, body weight, or body fat percentage were observed compared to baseline values.	Talbott *et al.*, 2013
75 men with history of infertility (average age of 32.7 years)	100 mg Tongkat Ali Root Extract twice daily (total of 200 mg/day) or placebo for three 3-month cycles	• Sperm quality (sperm concentration, motility and morphology) • Achievement of pregnancy of female partners	None.	Tambi and Imran, 2010
31 women (ages 45 to 49)	100 mg/day Tongkat Ali Root Extract or placebo for 12 weeks	• Muscular strength (bench press, peg press, hand grip) • Power (vertical jump) • Balance • Flexibility • Endurance • Muscle size	None.	Yusof *et al.*, 2009

Table XIII.C.2-1	**Other Clinical Studies Conducted on Tongkat Ali Root Extract**			
Study Population	**Dose and Duration of Tongkat Ali Root Extract Treatment**	**Parameters Examined (as reported)**	**Relevant Findings Regarding Safety**	**Reference**
40 healthy men (mean age of 45 years)	75 mg, 4 times daily (total of 300 mg/day) of Tongkat Ali Root Extract or placebo for 12 weeks	• Physical fitness tests (sit and reach, hand grip, back and leg strength, sit up, push up) • Body composition • Ratio of hormonal values for testosterone-to-epitestosterone	This study was a subset of subjects already described by Ismail *et al.*, 2012. All safety parameters were published in Ismail *et al.*, 2012.	George *et al.*, 2013

XIII.D Allergenicity

Studies specifically examining the allergenic potential of this ingredient have not been conducted; however, the results of 3-month studies in humans do not suggest a potential for the development of sensitivities to this ingredient.

OVERALL CONCLUSION

Biotropics Malaysia Berhad intends to market Tongkat Ali Root Extract as a novel food ingredient in conventional food and beverage products and food supplements in the EU. Tongkat Ali Root Extract is a standardised water extract prepared from the dried grinded root chips of *E. longifolia*. Appropriate product specifications for identity and potential contaminants have been established for this ingredient, and the results of batch analyses indicate that the manufacturing process produces a consistent product free of heavy metal, microbial, pesticide, and aflatoxin contamination.

Tongkat Ali Root Extract is proposed for use as an ingredient in beverages (coffee, sports and energy drinks, tea-based beverages), chocolate and confectionary (chocolate bars, candies), cereal bars, products for special nutritional use (nutrition and energy bars) and food supplements (including capsule form, tablet form, and liquid form). The proposed use level in conventional food matrices is 50 mg per serving.

The potential intakes of Tongkat Ali Root Extract from its proposed uses in conventional foods were estimated using consumption data from the European Food Safety Authority (EFSA) Comprehensive Food Consumption Database utilising the Food Additive Intake Model (FAIM) tool. Based on the maximum proposed use of the ingredient, which was applied across the broad food categories established in the FAIM tool, the mean and heavy-level intakes of Tongkat Ali Root Extract from its uses in conventional foods were 0.8 to 5.9 mg/kg body weight/day and 3.3 to 12.1 mg/kg body weight/day, respectively, in the intended target population of adults. Among the non-target demographic that may be unintentionally exposed to Tongkat Ali Root Extract, the worst-case heavy-level intakes of the ingredient were estimated to be 3.0 to 11.1 mg/kg body weight/day in adolescents.

Estimates for the daily intake of Tongkat Ali Root Extract from individual proposed food-uses were further estimated using a more refined approach utilising data from the UK NDNS. Based on the maximum proposed use of the ingredient in each individual food categories of interest, the estimated all-user mean and 95th percentile intakes among the target population of consuming adults were 56.4 and 165.9 mg/person/day (equivalent to 0.75 and 2.26 mg/kg body weight/day), respectively. The results of the assessment indicate that the worst-case 95th percentile Tongkat Ali Root Extract intakes for the non-target teenager population group was 150.1 mg/day (equivalent to 3.01 mg/kg body weight/day).

In addition to the proposed uses of the ingredient in conventional foods, Tongkat Ali Root Extract also is intended to be used as an ingredient in food supplements marketed to adult men and women. The maximum proposed use level of Tongkat Ali Root Extract in food supplements is 200 mg per day. Food supplements containing Tongkat Ali Root Extract would be consumed as an alternative source of Tongkat Ali Root Extract in conventional food forms and will be conspicuously labelled as such; therefore, it is not expected that individuals will consume both supplements and foods containing Tongkat Ali Root Extract.

The safety of Tongkat Ali Root Extract is supported by the results of traditional preclinical toxicological studies in animals in which a NOAEL of 1,000 mg/kg body weight/day, the highest dose tested, was derived in studies with durations of 28 days, 90 days, or 1 year. Based on the results of a reproductive and developmental toxicity screening study, Tongkat Ali Root Extract is not anticipated to have adverse effects on reproductive or developmental endpoints. Furthermore, the results of human studies in which the ingredient is consumed for durations of up to 2 months have indicated that no adverse health effects are anticipated with the consumption of Tongkat Ali Root Extract at up to 400 mg/day. Longer-term studies in which Tongkat Ali Root Extract has been provided for up to 12 weeks did not result in reports of sensitivities or intolerances at doses of up to 300 mg/day. Thus, given that the 95th percentile intakes of Tongkat Ali Root Extract from its proposed uses in conventional foods for the target population in adults (based on the refined assessment using the UK NDNS) is 2.26 mg/kg body weight/day, there exists a 442-fold safety factor compared to the NOAEL of 1,000 mg/kg body weight/day, the highest dose tested. In the non-target population, a worst-case 95th percentile intakes of 3.01 mg/kg body weight/day (in teenagers) was estimated, assuming that 100% of foods in which Tongkat Ali Root Extract is proposed for use contained the ingredient at the maximum use level and that all these foods were consumed by the non-target population. Even in this worst-case hypothetical scenario, the margin of safety remains large, at 332.

The safety of these dietary exposure estimates are further supported by clinical studies indicating that no adverse effects are observed in humans following consumption of up to 400 mg/day of this ingredient in food supplement form.

Collectively, the scientific evidence presented herein demonstrates that Biotropics' Tongkat Ali Root Extract would not produce adverse health effects on human health under the intended conditions of use in conventional foods and food supplements.

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