

**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***

SUMMARY OF THE DOSSIER

NON-CONFIDENTIAL

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Table of Contents

	Page
INTRODUCTION	3
I SPECIFICATIONS FOR TONGKAT ALI ROOT EXTRACT	4
I.A Identity	4
I.B Specifications.....	4
I.C Batch Analyses	5
I.D Contaminants.....	5
I.E Stability	5
II EFFECT OF THE PRODUCTION PROCESS APPLIED TO THE INGREDIENT	5
III HISTORY OF THE SOURCE ORGANISM – <i>Eurycoma longifolia</i>	6
IX INTAKE/EXTENT OF USE OF TONGKAT ALI ROOT EXTRACT	6
IX.A Intended Uses of Tongkat Ali Root Extract.....	6
IX.B Anticipated Daily Intakes of Tongkat Ali Root Extract.....	7
IX.B.1 Intake Estimates Based on the EFSA Comprehensive Food Consumption Database and FAIM Tool	8
IX.B.2 Intake Estimate Based on the UK National Diet and Nutrition Survey (UK NDNS).....	8
IX.B.3 Food Supplements	9
IX.B.4 Conclusions Regarding Intakes of Tongkat Ali Root Extract	10
X INFORMATION FROM PREVIOUS HUMAN EXPOSURE TO THE NOVEL FOOD OR ITS SOURCE	10
X.A Information from Previous Human Exposure.....	10
XI NUTRITIONAL INFORMATION ON TONGKAT ALI ROOT EXTRACT.....	11
XII MICROBIOLOGICAL INFORMATION ON TONGKAT ALI ROOT EXTRACT.....	11
XIII TOXICOLOGICAL INFORMATION ON TONGKAT ALI ROOT EXTRACT	11
XIII.A Absorption, Distribution, Metabolism, and Elimination (ADME)	11
XIII.B Toxicological Studies	12
XIII.B.1 Acute Toxicity Studies	12
XIII.B.2 Repeated Dose Toxicity Studies.....	12
XIII.B.3 Developmental and Reproductive Toxicity	13
XIII.B.4 Mutagenicity and Genotoxicity	14

SUMMARY OF THE DOSSIER (NON-CONFIDENTIAL)

XIII.C Human Studies	15
XIII.D Allergenicity	17
REFERENCES	17

List of Tables

Table I.B-1	Specifications Established for Tongkat Ali Root Extract.....	4
Table IX.A-1	Summary of the Individual Proposed Food Uses and Use Levels for Tongkat Ali Root Extract in the European Union.....	7
Table IX.B.1-1	Summary of Estimated Intakes of Tongkat Ali Root Extract from Proposed Food Uses Using the EFSA FAIM Tool	8
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)	9
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)	9
Table X.A-1	Countries in Which Products Containing Tongkat Ali Root Extract are Currently Marketed.....	11
Table XIII.A-1	Pharmacokinetic Values for the Bioactive Component of Tongkat Ali Root Extract, Eurycomanone (Low <i>et al.</i> , 2005).....	12

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

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, a 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. 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".

I SPECIFICATIONS FOR TONGKAT ALI ROOT EXTRACT

I.A Identity

Tongkat Ali Root Extract is a standardised water extract prepared from the dried grounded root chips of *Eurycoma longifolia*. The characteristic compound, erycomanone, is identified by the CAS number 84633-29-4. Tongkat Ali Root Extract is standardised according to the specifications established in the Malaysian Standard for this extract. The ingredient is characterised by chromatographic methods and may be further identified by matrix-assisted laser desorption/ionisation (MALDI) with time of flight (TOF) detection.

I.B Specifications

The proposed ingredient 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	Internal methods
Odour	Characteristic	Internal methods
Flavour	Bitter	Internal methods
Form/texture	Fine powder	Internal methods
Extraneous material	Free from foreign matter	Internal methods
Physical Characteristics		
Moisture content (%)	< 8.0	Loss on drying
Average mesh size	90% smaller than 100 mesh	Internal methods
Bioactive content		
Eurycomanone (%)	0.8 to 1.5	Internal methods
Total protein (%)	22 to 45	Lowry Method
Total polysaccharide (%)	30 to 55	Internal methods
Glycosaponin (%)	40 to 65	Gravimetric method
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
<i>Salmonella</i> (/10g)	Absent	Ph. Eu. 2.6.12
<i>Escherichia coli</i> (/1g)	Absent	Ph. Eu. 2.6.12

SUMMARY OF THE DOSSIER (NON-CONFIDENTIAL)

Parameter	Specifications	Methods
<i>Staphylococcus aureus</i> (/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

The results of analyses of 3 non-consecutive batches of Tongkat Ali Root Extract verify that the manufacturing process for Tongkat Ali Root Extract yields a consistent product that complies with established specifications.

Proximate analyses also have been conducted on samples of Tongkat Ali Root Extract and the results indicate that the ingredient is comprised mainly of carbohydrates and proteins, with negligible levels of fat.

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 indicate that the ingredient is free of these contaminants.

I.E Stability

Based on real-time and accelerated stability testing conducted on the ingredient, 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.

II EFFECT OF THE PRODUCTION PROCESS APPLIED TO THE INGREDIENT

Tongkat Ali Root Extract is a water extract of the dried grounded roots of *E. longifolia*, which is dried and prepared in a fine powder form. The methods employed are typical of the food industry. It is not anticipated that any toxicological, nutritional, or microbiological hazards will arise from the 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.

III HISTORY OF THE SOURCE ORGANISM – *Eurycoma longifolia*

Biotropics' Tongkat Ali Root Extract ingredient is produced from the dried roots of *Eurycoma longifolia* (common name Tongkat ali; Malaysian ginseng), 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.

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

Kingdom:	Plantae
Order	Sapindales
Family	Simaroubaceae
Genus	Eurycoma
Species	longifolia

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.

IX INTAKE/EXTENT OF USE OF TONGKAT ALI ROOT EXTRACT

IX.A Intended Uses of Tongkat Ali Root Extract

Tongkat Ali Root Extract is proposed for use as an ingredient in beverages, chocolate and confectionary, cereal bars, products for special nutritional use, and food supplements (see Table IX.A-1). The proposed use level in these foods is from 50 to 75 mg per serving. 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. The marketing of food and food supplements containing Tongkat Ali Root Extract will not be restricted geographically and is not intended to replace other foods currently in on the market.

SUMMARY OF THE DOSSIER (NON-CONFIDENTIAL)

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.

SUMMARY OF THE DOSSIER (NON-CONFIDENTIAL)

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

The results of the intakes assessment using the EFSA FAIM tool, 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. Intakes in adolescents (aged 10 to 17 years) and the elderly (aged 65 years and older) were comparable to those reported in adults. 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 (data not shown).

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

An additional exposure assessment was performed using data on the UK population from the NDNS Rolling Survey Programme (Department of Health, 2014; UKDA, 2014). Mean and high percentile estimates for the intake of Tongkat Ali Root Extract by the UK population (teenagers, adults, and the elderly¹) were generated on an absolute basis (Table IX.B.2-1) and on a per body weight basis (Table IX.B.2-2).

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. 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.

¹ 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) were included as a worst-case scenario in which this population group may incidentally consume foods containing this ingredient.

SUMMARY OF THE DOSSIER (NON-CONFIDENTIAL)

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	95 th Percentile	%	n	Mean	95 th 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	95 th Percentile	%	n	Mean	95 th 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.

The total adult UK population was identified as being significant consumers of chocolate bars (38.0%) and coffee-based drinks (11.7%) (data not shown). 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.

SUMMARY OF THE DOSSIER (NON-CONFIDENTIAL)

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.

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

X.A Information from Previous Human Exposure

Although there is no significant history of consumption of the ingredient in the Community, 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.

SUMMARY OF THE DOSSIER (NON-CONFIDENTIAL)

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

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. Based on the results of proximate analyses and elemental composition of the ingredient, 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.

XII MICROBIOLOGICAL INFORMATION ON TONGKAT ALI ROOT EXTRACT

The product specifications for Tongkat Ali Root Extract ensure the final ingredient is free from microbiological contaminants and the results of microbiological analyses confirm the absence of bacteria, yeast, and mould even after 36 months of storage.

XIII TOXICOLOGICAL INFORMATION ON TONGKAT ALI ROOT EXTRACT

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). The pharmacokinetic values that were determined following intravenous and oral administration of the ingredient in male Sprague-Dawley rats are summarised in Table XIII.A-1.

SUMMARY OF THE DOSSIER (NON-CONFIDENTIAL)

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

Abbreviations: - = not available. Values are presented as mean \pm 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

The toxicological properties of Tongkat Ali Root Extract have been evaluated through traditional pre-clinical safety studies, as well as several human studies. A brief summary of the main findings is provided below.

XIII.B.1 Acute Toxicity Studies

The results of an acute oral toxicity study conducted in Wistar rats indicate that the oral median lethal dose (LD_{50}) of Tongkat Ali Root Extract is greater than 2,000 mg/kg body weight (Choudhary *et al.*, 2012). Therefore, Tongkat Ali Root Extract is not considered to be an acute oral toxicant.

XIII.B.2 Repeated Dose Toxicity Studies

The potential for oral toxicity following repeated exposures was examined in a sub-acute repeat dose oral toxicity study was conducted with Tongkat Ali Root Extract (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. 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

SUMMARY OF THE DOSSIER (NON-CONFIDENTIAL)

1,000 mg/kg body weight/day, the highest dose tested, was established for both male and female rats.

The safety of Tongkat Ali Root Extract also was examined in a sub-chronic repeat dose oral toxicity study (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. As with the 28-day oral toxicity study, 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.

A chronic repeat dose oral toxicity study also was conducted to examine the safety of Tongkat Ali Root Extract (Gohel, 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. Similar to the sub-acute and sub-chronic studies, no treatment related mortality or morbidity was observed. No toxicological relevant or treatment related clinical signs of toxicity, ophthalmological abnormalities, functional observational battery observations, or clinical and neurobehavioural observations were reported. Although incidental effects were observed at haematology, clinical chemistry, urinalysis, and organ weight examination, these effects were considered to be adaptive and not associated with any histological lesions. As such, they were not considered toxicologically relevant. 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

The reproductive and developmental toxicity of Tongkat Ali Root Extract was evaluated in a reproductive and developmental toxicity screening study (unpublished study report). 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. No mortalities occurred during the treatment period. Although some clinical signs were noted in rats receiving 500 and 1,000 mg/kg body weight/day and statistically significant differences in body weight gain in association with changes in food consumption were noted at certain periods of the treatment phase, it was

SUMMARY OF THE DOSSIER (NON-CONFIDENTIAL)

concluded that no dose relationship could be established, effects were transient, and thus, these observed differences were considered toxicologically not relevant. 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.

The effect of Tongkat Ali Root Extract on sperm has been evaluated in Sprague-Dawley rats. Male rats 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). 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 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 increases in 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

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 (unpublished study report). In the experiment, *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535, and TA1537 were exposed to Tongkat Ali Root Extract using the standard plate incorporation method at concentrations of up to 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.

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 (unpublished study report). 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

SUMMARY OF THE DOSSIER (NON-CONFIDENTIAL)

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.

To follow up the *in vitro* assessments, an *in vivo* mouse erythrocyte micronucleus assay was conducted (unpublished study report). NMRI mice were administered a single dose of 0 (negative control), 100, 250, or 500 mg/kg body weight by intraperitoneal injection. No significant differences in the ratios of polychromatic erythrocytes to normochromatic erythrocytes were observed between groups 24 hours post-administration and no increases in the frequency of micronucleated polychromatic erythrocytes were observed compared to the negative control.

Taking into consideration the results of the *in vitro* and *in vivo* genotoxicity studies, Tongkat Ali Root Extract is not considered to have genotoxic potential.

XIII.C Human Studies

The effects of oral administration of Tongkat Ali Root Extract on supporting men’s health have been evaluated in several studies. In particular, two studies have examined safety endpoints and are discussed in further detail herein.

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 illnesses² (ages 30 to 55 years) were provided capsules containing 75 mg of Tongkat Ali Root Extract 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 phosphatase, 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;

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

SUMMARY OF THE DOSSIER (NON-CONFIDENTIAL)

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, 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 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 ranges³. 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.”

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

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.

SUMMARY OF THE DOSSIER (NON-CONFIDENTIAL)

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 (Hamzah and Yusof, 2003; Talbott *et al.*, 2006, 2013; Yusof *et al.*, 2009; Tambi and Imran, 2010; 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.

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