Application for the Approval of Oligonol®

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

08 January 2014

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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 approval of Oligonol® as a food ingredient in conventional foods and food supplements (European Parliament and the Council of the European Union, 1997). Oligonol® is manufactured from a 5:1 mixture of extracts from the lychee fruit (*Litchi chinensis* Sonn.) and green tea leaves [*Camellia sinensis* (L.) Kuntze], which undergoes an oligomerisation process whereby the polyphenols present are cleaved into monomers and lower molecular weight oligomers. Polyphenols account for >80% of the Oligonol® product; specifically, Oligonol® is comprised mainly of monomeric flavan-3-ols, as well as procyanidins formed from the condensation of these monomeric units. Although the polyphenols present in Oligonol® are naturally present in the diet, Oligonol® itself does not have a history of use in foods or food supplements for the general population in the European Union (EU) prior to 15 May 1997. Therefore, Oligonol® falls under category (e) of Article 1(2) of 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. Following Recommendation 97/618/EC (Commission of the European Communities, 1997), Oligonol® also belongs to Class 2 “complex NF from non-GM source” and sub-class (1) “the source of the novel food has a history of food use in the Community”.

To manufacture Oligonol®, extracts of the lychee fruit and green tea leaves are blended in a ratio of 5:1, and the polyphenols present are oligomerised *via* a de-polymerisation reaction. Oligonol® is manufactured in accordance with Good Manufacturing Practice (GMP) for dietary supplements, a standard that is based on guidelines prepared by the Japan Ministry of Health, Labour and Welfare and is certified by the Japan Health and Nutrition Food Association. Oligonol® is also manufactured in accordance with ISO 9001:2008 and ISO 22000:2005. Analysis of 3 non-consecutive lots of Oligonol® demonstrates that the manufacturing process produces a consistent product meeting physical, chemical, and microbiological specifications. In addition, stability tests have demonstrated the stability of Oligonol® when stored in bulk and under the conditions of its intended uses.

Oligonol® is proposed for use in a variety of conventional foods and beverages at use levels ranging from 100 to 1,150 mg/kg or mg/L. Using the European Food Safety Authority (EFSA) ‘Food Additives Intake Model’ (FAIM) tool, the mean daily estimated intake of

Oligonol® from the proposed uses by the EU population was estimated to range from 3.3 to14.3 mg/kg body weight/day in toddlers, 4.2 to 14.6 mg/kg body weight/day in children, 2.5 to 6.7 mg/kg body weight/day in adolescents, 1.7 to 8.8 mg/kg body weight/day in adults, and 1.4 to 7.0 mg/kg body weight/day in elderly individuals. "Heavy level" intakes ranged from 11.1 to 27.1 mg/kg body weight/day in toddlers, 9.1 to 25.4 mg/kg body weight/day in children, 4.2 to 13.7 mg/kg body weight/day in adolescents, 3.1 to 15.5 mg/kg body weight/day in adults, and 2.8 to 12.6 mg/kg body weight/day in elderly individuals. Using the reference body weights for the EU population, the mean daily estimated intake of Oligonol® from the proposed uses correspond to 40 to 172 mg/day in toddlers, 97 to 336 mg/day in children, 108 to 288 mg/day in adolescents (10 to 14 years old), 153 to 409 mg/day in adolescents (14 to 18 years old), 119 to 616 mg/day in adults, and 98 to 490 mg/day in elderly individuals. "Heavy level" intakes correspond to 133 to 325 mg/day in toddlers, 209 to 584 mg/day in children, 181 to 589 mg/day in adolescents (10 to 14 years old), 256 to 836 mg/day in adolescents (14 to 18 years old), 217 to 1,085 mg/day in adults, and 196 to 882 mg/day in elderly individuals.

Oligonol® is also proposed for use in food supplements at 200 mg/day. Given that Oligonol® food supplements are intended to serve as an alternative to foods enriched with Oligonol®, it is highly unlikely that individuals will consume both Oligonol® food supplements and Oligonol®-containing foods. Under the worst-case scenario where Oligonol® food supplements are also consumed by individuals with the highest estimated "heavy level" intake of Oligonol® from the proposed food uses, the total exposure to Oligonol® is estimated at 1,285 mg/day (18.4 mg/kg body weight/day).

Many dietary sources, including fruits, vegetables, and tea, are rich in polyphenolic compounds. Information on the metabolic fate of orally administered Oligonol® can be extrapolated from studies conducted with its monomeric and oligomeric polyphenol constituents. Animal and human studies provide evidence that monomers are absorbed to a greater extent than the oligomeric procyanidins. Flavonoids that are absorbed are transported to the liver *via* the portal system, where they can be conjugated through glucuronidation, sulfation, or methylation and eliminated. Monomers and procyanidins that are not absorbed in the gastrointestinal tract may be partially metabolised by the intestinal microflora to phenylvalerolactones and phenolic acids.

Both lychee fruit and green tea leaves, which are used as the source of polyphenols for the manufacture of Oligonol®, have an extensive history of safe consumption in the diet. The safety of Oligonol® is also supported by the results of several product-specific toxicological studies. Oligonol® is of low acute toxicity, with the median lethal dose determined to be greater than 2,000 mg/kg in both sexes of Sprague-Dawley rats following oral administration. The sub-chronic toxicity of orally administered Oligonol® has been evaluated in two 90-day gavage studies conducted in rats and one 90-day feeding study in mice. No toxicologically relevant effects were reported in two 90-day studies where Sprague-Dawley rats were administered Oligonol® at doses up to 1,000 mg/kg body weight/day by gavage. There were no treatment-related changes in body weight, food consumption, and haematology, clinical chemistry, and urinalysis parameters. The no-observed-adverse-effect level (NOAEL) of

Oligonol® was determined to be 1,000 mg/kg body weight/day, the highest dose tested, for both males and females. These findings were further corroborated by a lack of toxicity observed in a 90-day feeding study conducted in mice, which administered doses of Oligonol® at up to 200 mg/kg body weight/day. Oligonol® was not mutagenic/genotoxic when evaluated using a series of *in vitro* and *in vivo* assays. Oligonol® was also well tolerated in several clinical studies, with no adverse events reported and no treatment-related changes in haematology or serum biochemistry parameters observed in subjects consuming 200 to 600 mg/day of Oligonol® for as long as 3 months. Data from oral toxicity studies (*i.e.*, acute and short-term) and human clinical studies conducted with an Oligonol®-like product, which is manufactured using the same oligomerisation process (but from different starting materials) and contains similar polyphenolic composition as Oligonol®, can be used to further corroborate the safety of Oligonol®.

Based on a NOAEL of 1,000 mg/kg body weight/day derived from product-specific 90-day oral toxicity studies in rats, the safety margin in adults is at least 114-fold for the estimated mean intakes of Oligonol® (1.7 to 8.8 mg/kg body weight/day), and at least 65-fold for the estimated "heavy level" intakes of Oligonol® (3.1 to 15.5 mg/kg body weight/day) from the proposed food uses of Oligonol®. Oligonol® is also proposed for use in food supplements; there is a 54-fold safety margin for the total exposure to Oligonol® estimated under the worst-case scenario where Oligonol® food supplements are consumed at the maximum recommended levels by adults with the highest estimated "heavy level" intake of Oligonol® from the proposed food uses (*i.e.*, 18.4 mg/kg body weight/day). Although some of the safety margins are less than the generally accepted level of 100-fold, it is important to note that these safety margins are the worst-case scenario estimates. Considerable variation was observed in the exposure estimates derived using the EFSA FAIM Tool (*i.e.*, approximately 3- to 5-fold difference), and the safety margins were calculated using the highest intake levels of Oligonol® observed across a range reported based on data from various dietary surveys collected in 17 different European countries. The exposure estimates are also highly conservative as it is assumed that all foods containing Oligonol® are consumed at the maximum proposed use levels; therefore, the actual intake of Oligonol® from the proposed conditions of use will be less than anticipated. Additionally, it is very unlikely that individuals with the highest estimated "heavy level" intake of Oligonol® from food uses would seek to further increase their dietary intakes by also consuming food supplements containing Oligonol®. As such, the total exposure estimates for the consumption of both food supplements and foods containing Oligonol® are likely to be grossly overestimated. In light of these caveats, and the fact that the NOAEL of 1,000 mg/kg body weight/day represents the highest dose tested in product-specific 90-day toxicity studies, the proposed food and food supplement uses of Oligonol® can be justified.

Although green tea has a long history of safe consumption, there have been some concerns raised (*e.g.*, liver toxicity) over the safety of highly concentrated, purified forms of green tea extracts being marketed primarily as dietary supplements for weight loss purposes. Since green tea catechins are a minor constituent of Oligonol® (accounting for approximately 16% of the product), Amino Up undertook a thorough evaluation of the literature pertaining to the

adverse effects of green tea extracts to determine their implication, if any, on the safety of Oligonol®. Although evidence of liver toxicity, along with the novel finding of nasal toxicity, were observed in a 14-week oral toxicity study conducted by the National Toxicology Program (NTP) where a specific green tea extract preparation was administered by gavage to rodents, these findings are not considered relevant to the safety of Oligonol®. No evidence of liver toxicity were observed in two 90-day oral toxicity studies conducted in rats where Oligonol® was administered at doses up to 1,000 mg/kg body weight/day, and 3 human studies did not indicate any changes in liver function in subjects consuming Oligonol® at doses up to 600 mg/day. Furthermore, in another subchronic study conducted in rats, no histopathological changes to the nasal cavity were observed following administration of Oligonol® by gavage at dosages up to 1,000 mg/body weight/day for 90 days, thereby confirming that the effects noted in the 14-week toxicity studies conducted by the NTP were not relevant to Oligonol®. Lastly, it is important to note that the amount of green tea catechins that would be consumed from the proposed uses of Oligonol® is comparable to the amount obtained from one 200 mL serving of green tea. As such, the green tea extract component of Oligonol® is not expected to pose safety concerns under its proposed conditions of use.

Overall, the scientific evidence presented demonstrates that Oligonol® would not produce adverse effects on human health under the intended conditions of use in conventional foods and food supplements in the EU.

GENERAL INTRODUCTION

Amino Up Chemical Company Ltd. (Amino Up) proposes to market Oligonol®, a 5:1 mixture of extracts from lychee fruit (*Litchi chinensis* Sonn.) and green tea leaves (*Camellia sinensis*), as an ingredient in conventional foods and food supplements in Europe. Oligonol® is manufactured by an oligomerisation reaction that cleaves the polyphenols in present in the lychee fruit extract and green tea leaf extract into monomers and low molecular weight oligomers. Specifically, Oligonol® is composed mainly of monomeric flavan-3-ols, as well as procyanidins formed from the condensation of these monomeric units. Although the polyphenols present in Oligonol® occur naturally in the diet, Oligonol® does not have a history of use in foods or food supplements for the general population in the European Union (EU) prior to 15 May 1997. Additionally, Oligonol® is considered a novel food/food ingredient since it is an isolated product from its source (*i.e.*, lychee fruit and green tea leaves). Therefore, 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.

In accordance with the categories defined by Article 1(2) of Regulation (EC) No 258/97 (European Parliament and the Council of the European Union, 1997), Oligonol® falls under the following category:

> (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.

An application for placing a novel food ingredient on the market in the EU is required to follow the European Commission's Scientific Committee on Food (SCF) Recommendation 97/618/EC (Commission of the European Communities, 1997). Under Section 4 of this Recommendation pertaining to the "Scientific Classification of Novel Foods for the Assessment of Wholesomeness", Oligonol® belongs to Class 2: "complex novel food/novel food ingredients (NF) from non-genetically modified source" and sub-class (1): "the source of the NF has a history of food use in the Community". The dossier presented herein follows the structured sections which are required to establish the safety of a Class 2(1) novel food ingredient:

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
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. As outlined below in Sections I through XIII, the required questions are identified and subsequently addressed with the appropriate data. Note that Categories IV to VIII defined in the SCF Recommendations does not need to be included for an application of a Class 2(1) novel food ingredient.

A glossary is provided at the end of the document to explain the abbreviated terms referred to in the dossier.

I SPECIFICATION OF OLIGONOL®

Based on the SCF guidelines, the following questions must be answered in the affirmative to ensure sufficient information pertaining to the specifications of the novel food:

- “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?”
- “Is the information representative of the novel food when produced on a commercial scale?”
- “Is appropriate analytical information available on potentially toxic inherent constituents, external contaminants and nutrients?”

These questions have been addressed in Sections I.A through I.D.

I.A Identity of Oligonol®

I.A.1 Common Name or Usual Name

Polyphenols derived from lychee fruit (*Litchi chinensis* Sonn.) and green tea leaves [*Camellia sinensis* (L.) Kuntze]

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

Not applicable.

I.A.3 Chemical Composition

I.A.3.1 Compositional Analyses of Oligonol®

Oligonol® is a reddish-brown powder composed of a 5:1 mixture of powdered extracts from lychee fruit (*L. chinensis* Sonn.) and green tea leaves [*C. sinensis* (L.) Kuntze], which undergoes an oligomerisation reaction to cleave the polyphenols present in these starting materials into lower molecular weight oligomers and monomers (Tanaka *et al.*, 2007). The compositional analysis of three non-consecutive sample lots of Oligonol® is presented in Table I.A.3.1-1. The total polyphenolic content of Oligonol® is measurable using the Folin-Denis assay and accounts for >80% of the total mixture. Oligonol® consists mainly of monomeric flavan-3-ols, as well as procyanidins formed from the condensation of these monomeric units. Specifically, Oligonol® contains four different flavan-3-ol monomers, (+)-catechin, (-)-epicatechin (EC), (-)-epicatechin-3-gallate (ECG), and (-)-epigallocatechin gallate (EGCG), which constitute approximately 16% of Oligonol® combined. In addition, 5 polyphenol dimers [procyanidin A1, procyanidin A2, procyanidin B1, procyanidin B2, and (-)-epicatechin-(4β→8)-(-)-epigallocatechin gallate] and a trimer [(-)-epicatechin-(4β→8, 2β→O→7)-epicatechin-(4β→8)-epicatechin] have been identified in Oligonol® and quantified using high-performance liquid chromatography (HPLC). The combined dimers and trimer content constitute approximately 14 and 4% of Oligonol®, respectively. Longer oligomers, composed of varying combinations of monomers, also are present in Oligonol®; however, due to technical limitations, these cannot be readily identified or quantified.

Table I.A.3.1-1 Compositional Analysis of Three Sample Lots of Oligonol®				
Constituents		**Lot Number[a]**		
		OLF0703	**OLF0705**	**OLF0804**
Material Balance Analysis[b]				
Monomeric Flavan-3-ols + Procyanidins		**92.5**	**95.6**	**92.7**
Sugars (%)		3.5	3.7	3.5
Moisture (%)		0.0	0.0	0.0
Protein (%)		1.6	1.6	1.6
Total fat (%)		0.5	0.6	1.0
Ash (%)		0.2	0.3	0.2
Total (%)		**98.3**	**101.8**	**99.0**
Phenolic Composition				
Monomeric Flavan-3-ols (%)				
(+)-Catechin and (-)-Epicatechin		8.2	7.9	6.4
(-)-Epicatechin-3-gallate (ECG)		2.1	2.0	2.3
(-)-Epigallocatechin gallate (ECGC)		6.0	6.2	6.4
Total Monomeric Flavan-3-ols (%)		**16.3**	**16.1**	**15.1**
Procyanidins (%)				
Dimers	Procyanidin A1	4.1	4.0	3.5
	Procyanidin A2	5.0	4.9	5.3
	Procyanidin B1	1.3	1.3	0.4
	Procyanidin B2	3.1	3.0	2.5
	(-)-Epicatechin-(4β→8)-(-)-epigallocatechin gallate	0.3	0.3	0.5
	Total Dimers (%)	13.8	13.5	12.2
Trimer	(-)-Epicatechin-(4β→8, 2β→O→7)-epicatechin-(4β→8)-epicatechin	3.8	3.8	1.9
Other procyanidins	Not further classified	58.6	62.2	63.5
Total Procyanidins (%)		**76.2**	**79.5**	**77.6**
Total Monomeric Flavan-3-ols + Procyanidins		**92.5**	**95.6**	**92.7**

[a] Analyses were conducted on freeze-dried samples of Oligonol®

[b] Protein content was determined by Kjeldahl nitrogen analysis (16th Edition of the Japanese Pharmacopeia), total fat content was determined by acidolysis (Japan Food Hygiene Association Method), and ash content was determined by methods in the 16th Edition of the Japanese Pharmacopeia (Appendix A). Sugar content was determined as the balance-total weight with other components subtracted. The HPLC methods used to identify and quantify the polyphenolic constituents of Oligonol® is also provided in Appendix A.

I.A.3.2 Characterisation of the Monomeric, Dimeric, and Trimeric Constituents of Oligonol®

Oligonol® contains 4 different flavan-3-ol monomers, including (+)-catechin, EC, ECG, and EGCG at a combined level of approximately 16%. In addition, 5 polyphenol dimers [procyanidin A1, procyanidin A2, procyanidin B1, procyanidin B2, and (-)-epicatechin-(4β→8)-(-)-epigallocatechin gallate] and a trimer [(-)-epicatechin-(4β→8, 2β→O→7)-epicatechin-(4β→8)-epicatechin] have been identified in Oligonol®. The combined dimers and trimer constitute approximately 14 and 4% of Oligonol®, respectively. The CAS numbers, structural and empirical formulae, molecular weights, and synonyms of these polyphenolic constituents are summarised in Table I.A.3.2-1 and Table I.A.3.2-2.

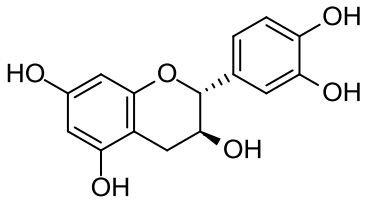
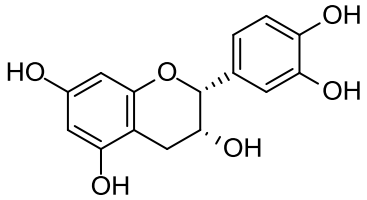
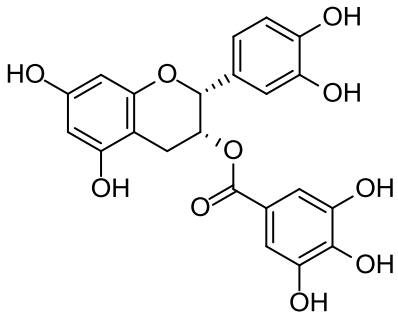
Table I.A.3.2-1 Characteristics of the Monomers Present in Oligonol®					
Monomer	**Structure**	**Synonyms**	**CAS#**	**Formula**	**Molecular Wt**
(+)-Catechin		catechuic acid; cianidanol; dexcyanidanolcyanidol; (+)-cyanidanol-3; catechol; catechin; 3,3',4',5,7-flavanpentol; catechinic acid; catergen Systematic Name: (2R-trans)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-1-benzopyran-3,5,7-triol;	154-23-4	$C_{15}H_{14}O_6$	290.27
(-)-Epicatechin (EC)		L-epicatechin Systematic Name: 2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-1-benzopyran-3,5,7-triol	490-46-0	$C_{15}H_{14}O_6$	290.27
(-)-Epicatechin-3-gallate (ECG)		(-)-epicatechin-3-O-gallate; epicatechin gallate; L-epicatechin gallate Systematic Name: Benzoic acid, 3,4,5-trihydroxy-,(2R,3R)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-5,7-dihydroxy-2H-1-benzopyran-3-yl ester	1257-08-5	$C_{22}H_{18}O_{10}$	442.37

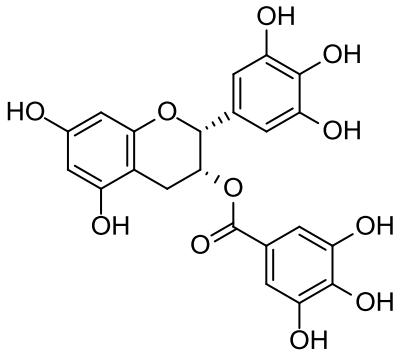
Table I.A.3.2-1 Characteristics of the Monomers Present in Oligonol®					
Monomer	**Structure**	**Synonyms**	**CAS#**	**Formula**	**Molecular Wt**
(-)-Epigallocatechin gallate (EGCG)		(-)-epigallocatechin 3-O-gallate; (-)-epigallocatechol gallate; epigallocatechin 3-gallate; epigallocatechin gallate Systematic Name: 3,4,5-trihydroxybenzoic acid; (2R-cis)-3,4-dihydro-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-2H-1-benzopyran-3-yl ester	989-51-5	$C_{22}H_{18}O_{11}$	458.38

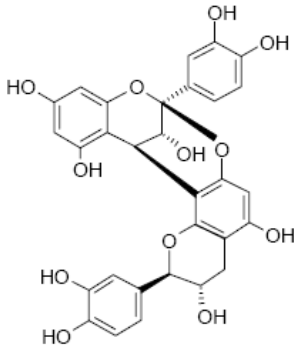
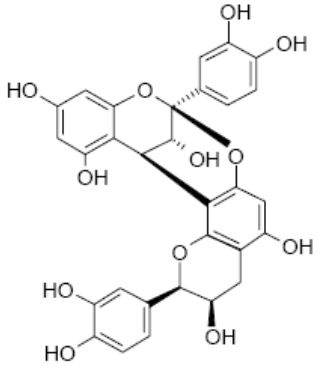
Table I.A.3.2-2 Characteristics of the Oligomers Present in Oligonol®					
Oligomer	**Structure**	**Synonyms**	**CAS#**	**Formula**	**Molecular Wt**
Procyanidin A1		Proanthocyanidin A1 Systematic name: (2R,3S,8S,14R,15R)-2,8-bis(3,4-dihydroxyphenyl)-2,3,4,14-tetrahydro-8,14-methanobenzo[7,8][1,3]dioxocino[4,5-h]chromene-3,5,11,13,15-pentaol	12798-56-0	$C_{30}H_{24}O_{12}$	576.51
Procyanidin A2		Proanthocyanidin A2 Systematic name: 2,14-Bis(3,4-dihydroxyphenyl)-3,4-dihydro-8,14-methano-2H,8H,14H-1-benzopyrano(8,7-c)(1,5)benzodioxocin-3,5,9,11,15-pentol	41743-41-3	$C_{30}H_{24}O_{12}$	576.51

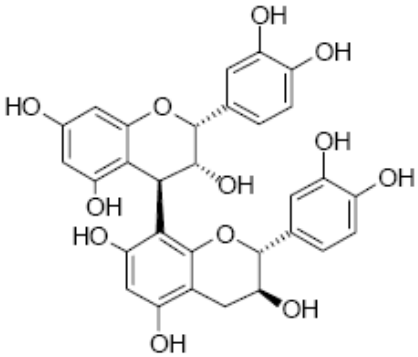
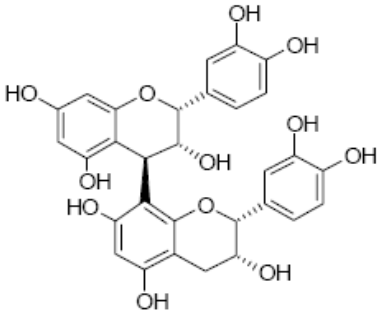
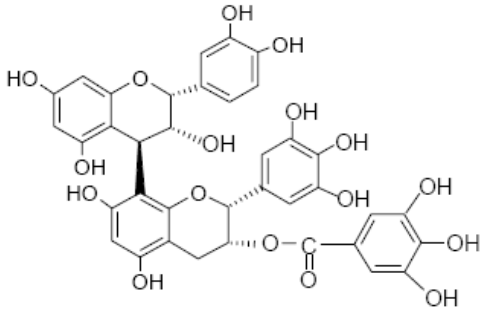
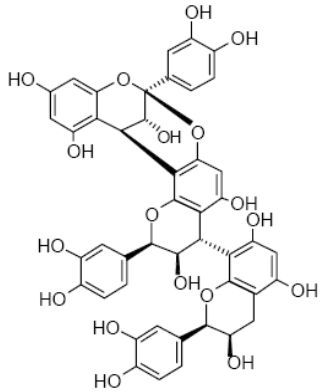
Table I.A.3.2-2 Characteristics of the Oligomers Present in Oligonol®					
Oligomer	**Structure**	**Synonyms**	**CAS#**	**Formula**	**Molecular Wt**
Procyanidin B1		Proanthocyanidin B1 Systematic name: 2,2'bis(3,4-dihydroxyphenyl)-3,3'4,4'-tetrahydro-(4,8-Bi-2H-1-benzopyran)-3,3',5,5',7,7'-hexol	20315-25-7	$C_{30}H_{26}O_{12}$	578.53
Procyanidin B2		Proanthocyanidin B2 Systematic name: (2R-(2alpha, 3alpha, 4beta(2'R*, 3'R*)))-2,2'-Bis(3,4-dihydroxyphenyl)-3,3',4,4'-tetrahydro-(4,8'-Bi-2H-1-benzopyran)-3,3',5,5',7,7'-hexol;	29106-49-8	$C_{30}H_{26}O_{12}$	578.53
(-)-Epicatechin-(4β→8)-(-)-epigallocatechin gallate [EC-EGCG (Dimer consisting of the monomers (-)-epicatechin (EC) and (-)-epigallocatechin gallate (EGCG); see Table 2.3.1-1)]		None identified	Not identified	$C_{37}H_{30}O_{17}$	746.63

Table I.A.3.2-2 Characteristics of the Oligomers Present in Oligonol®					
Oligomer	**Structure**	**Synonyms**	**CAS#**	**Formula**	**Molecular Wt**
(-)-Epicatechin-(4β→8, 2β→O→7)-epicatechin-(4β→8)-epicatechin [Trimer consisting of the dimer procyanidin A2 and the monomer, EC]		None identified.	Not identified	$C_{45}H_{36}O_{18}$	864.77

I.B Product Specifications

The product specifications established for Oligonol® are presented in Table I.B-1.

Table 1.B-1 Product Specifications for Oligonol®

Specification Parameter	Specification	Reference/Test Methodology Performance of Test
Identity		
Characteristic	Reddish-brown powder, Astringent taste	Sensory analysis
Moisture (%)	Not more than 5.0	Oven drying at 70°C for 6h under reduced pressure
Total Procyanidin (%)	More than 70	Porter method[a]
Monomeric Flavan-3-ols (%)	More than 10	HPLC method[a]
Heavy Metals		
Lead (Pb) (ppm)	Not more than 0.2	Atomic absorption spectrophotometry[b]
Arsenic (as As_2O_3) (ppm)	Not more than 1.0	Colorimetric method (arsenic limit test)[b]
Microbial Specifications		
Number of bacteria (CFU/g)	Not more than 1,000	Microbial Limit test (pour plate method)[b]
Mould and Yeast (CFU/g)	Not detected	Microbial Limit test (spread plate method)[b]
Coliforms (CFU/g)	Not detected	Microbial Limit test (spread plate method)[b]

Abbreviations: As_2O_3 = arsenic oxide; CFU = colony forming units; HPLC = high-performance liquid chromatography; ppm = parts per million.
[a] Refer to Appendix A-1 for descriptions of analytical methods
[b] Japanese Pharmacopeia (16^h edition) method. Refer to Appendix A-2 for details.

I.C Batch Analyses

Analysis of 3 non-consecutive lots of Oligonol® demonstrates that the manufacturing process produces a consistent product meeting physical, chemical, and microbiological specifications. The results of these batch analyses are presented in Table I.C-1. Certificates of Analysis are provided in Appendix B.

Table I.C-1 Summary of Product Analysis for 3 Non-Consecutive Lots of Oligonol®				
Specification Parameter	**Specification**	**Manufacturing Lot[a]**		
		OLF0703	**OLF0705**	**OLF0804**
Identity				
Characteristic	Reddish-brown powder, characteristic rough taste	Satisfied	Satisfied	Satisfied
Moisture (%)	Not more than 5.0	0.0	0.0	0.0
Total Procyanidin (%)	More than 70	76.2	79.5	77.6
Monomeric flavan-3-ols (%)	More than 10	16.3	16.1	15.1
Heavy Metals				
Lead (Pb) (ppm)	Not more than 0.2	Not detected	Not detected	Not detected
Arsenic (as As_2O_3) (ppm)	Not more than 1.0	≤1.0	≤1.0	≤1.0
Microbial Specifications				
Number of bacteria (CFU/g)	Not more than 1,000	0	0	0
Mould and yeast (CFU/g)	Not detected	Not detected	Not detected	Not detected
Coliforms (CFU/g)	Not detected	Not detected	Not detected	Not detected

Abbreviations: CFU = colony forming units; ppm = parts per million.
[a] Analyses were conducted on freeze-dried samples of Oligonol®

I.D Stability

I.D.1 Bulk Stability

The stability of the monomeric and polyphenol constituents of Oligonol® was assessed after one year of storage. A sample lot of the Oligonol® powder was stored at room temperature, in an aluminium bag to prevent light and moisture from affecting the sample. The levels of the flavan-3-ol monomers, [*i.e.*, (+)-catechin, EC, ECG, and EGCG], procyanidin dimers (procyanidin A1, A2, B1, B2, and EC-EGCG), and trimer (-)-epicatechin-(4β→8, 2β→*O*→7)-epicatechin-(4β→8)-epicatechin in the Oligonol® samples were measured at baseline and after 1 year of storage. No significant differences in the polyphenolic composition of Oligonol® were observed (see Table I.D.1-1). The study report is attached in Appendix C.

Table I.D.1-1 Composition of Monomeric Flavan-3-ol and Procyanidins in Oligonol® After One Year of Storage		
Component in Oligonol®	**Initial Composition (%)**	**Composition after 1 year (%)**
(+)-catechin and (-)-epicatechin	8.4	8.4
(-)-epicatechin-3-gallate	2.2	2.3
(-)-epigallocatechin gallate	6.2	6.3
Procyanidin A1	4.3	4.5
Procyanidin A2	5.2	5.2
Procyanidin B1	1.2	1.2
Procyanidin B2	2.6	3.0
(-)-epicatechin-(4β→8)-(-)-epigallocatechingallate	0.2	0.3
(-)-epicatechin-(4β→8, 2β→*O*→7)-epicatechin-(4β→8)-epicatechin	4.2	4.2
Total (%)	**34.5**	**35.4**

I.D.2 Stability under Intended Uses

The stability of Oligonol® in solutions with different pH levels following storage for 3 months was evaluated. Solutions of 50 ppm Oligonol® adjusted to pH of 3, 5, or 7 were stored at 4°C, room temperature, or 40°C. The Oligonol® solutions were analysed for total polyphenol content at study initiation, and at 1, 2, and 3 months of storage. The results of this stability study are summarised in Table I.D.2-1.

Oligonol® is proposed for use mainly in foods that are acidic (*e.g.*, fruit and vegetable-based beverages). Accordingly, the total polyphenol content of the Oligonol® was not altered for the duration of the storage period (up to 3 months) under acidic conditions (pH 3). Although Oligonol® will be less stable under alkaline conditions, such as dairy-based foods, these products are usually refrigerated, which would reduce the extent of degradation. Furthermore, as discussed in Section II.D.3, there are no safety concerns anticipated with the degradation products that may be formed from Oligonol® under alkaline conditions.

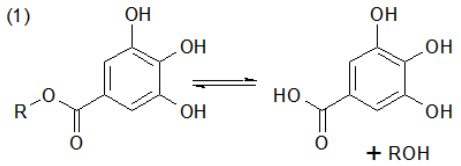
Table I.D.2-1 Total Polyphenol Content (%) in Oligonol® Following Storage at Various pH and Temperatures for Up to 3 Months				
pH	**Period**	**Storage Temperature**		
		4°C	**Room temperature**	**40°C**
3	At baseline	100	100	100
	After 1 month	100	100	100
	After 2 months	100	100	100
	After 3 months	100	100	100
5	At baseline	100	100	100
	After 1 month	100	98	80
	After 2 months	97	88	75
	After 3 months	96	88	71
7	At baseline	100	100	100
	After 1 month	100	93	62
	After 2 months	90	77	58
	After 3 months	85	72	45

A study was also conducted to investigate the sensitivity of Oligonol® to light. Oligonol®, at a concentration of 100 ppm in tap water, was maintained at 4°C either in the dark or exposed to light (3,000 to 3,500 lux) for 4 weeks. Total polyphenol content was measured at baseline, 2 and 4 weeks using the Folin-Denis method. No decline in total polyphenol content was observed under the dark or lit conditions.

I.D.3 Degradation Pathways and Products

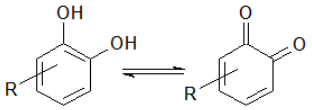
There are a number of well-established mechanisms by which the polyphenolic compounds found naturally in foods can degrade under conditions of high pH (>7), exposure to air and elevated temperatures (deMan, 1999; Francis, 1999). It is anticipated that similar processes would be observed with Oligonol®. Examples of these typical degradation processes are outlined below:

1. Hydrolysable systems such as (-)-epicatechin-3-gallate and (-)-epigallocatechin gallate may break down under alkaline conditions to release free gallic acid.



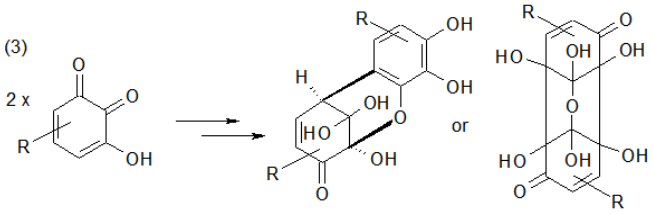
2. Oxidation of dihydroxybenzene systems results in the formation of quinones. In particular, alkaline hydrolysis of procyanidins may result in the formation of quinone intermediates which are then converted to anthocyanins.

(2)



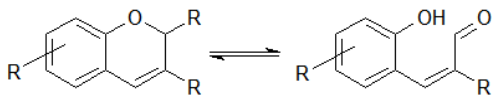
3. Oxidative coupling mechanisms will result in polymerisation to form polymers. In buffered solutions of pH 7.4, (-)epigallocatechin gallate forms the dimer theasinensin A by auto-oxidation (Tanaka *et al*., 2003).

(3)



4. Anthocyanins and some flavanoid derivatives may undergo ring opening under strongly alkaline conditions (pH 12) to form chalcones.

(4)



R = other substituents

The degradation products of Oligonol® under alkaline conditions will be the same to those found in other polyphenol-containing foods. For example, the oxidation products of procyanidins and the (-)-epigallocatechin gallate dimers (*i.e.*, theasinensins A and D) are constituents of black tea (Tanaka *et al.*, 2003). Background consumption of these degradation products from dietary sources are far greater those that may potentially arise from the proposed uses and use-levels of Oligonol®. Given the historical consumption of these degradation products in the diet, they are not considered to pose a safety concern to humans.

II EFFECT OF THE PRODUCTION PROCESS APPLIED TO OLIGONOL®

Based on the SCF guidelines, the following questions must be addressed to ensure sufficient information pertaining to the effect of the production process applied to the novel food:

- “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 collectively in Section II.A.

II.A Manufacturing Process

Oligonol® is manufactured an oligomerisation process whereby the polyphenols present in a 5:1 mixture of extracts from the lychee fruit (*Litchi chinensis* Sonn.) and green tea leaves [*Camellia sinensis* (L.) Kuntze] are cleaved into monomers and lower molecular weight oligomers. The starting materials and processing aids used in the manufacture of Oligonol® meet food-grade specifications. Furthermore, no pesticide residues were detected in the starting materials (*i.e.*, extracts of lychee fruit and green tea leaf) or Oligonol® that would be of toxicological concern. The production of Oligonol® is conducted in accordance with Good Manufacturing Practice (GMP) for dietary supplements, a standard that is based on guidelines prepared by the Japan Ministry of Health, Labour and Welfare and is certified by the Japan Health and Nutrition Food Association. Oligonol® is also manufactured in accordance with ISO 9001:2008 and ISO 22000:2005.

III HISTORY OF THE SOURCE ORGANISM OF OLIGONOL®

Based on the SCF guidelines, the following questions must be addressed to ensure sufficient information pertaining to the history of the source organism:

- “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 Sections III.A through III.C.

III.A Taxonomic Classification of the Source Organisms

Extracts of the lychee fruit (*L. chinensis* Sonn.) and leaves of the green tea plant [*C. sinensis* (L.) Kuntze] serve as the starting material for the production of Oligonol®. The taxonomic classification of the lychee fruit and green tea plant is provided in Table III.A-1.

Table III.A-1 Taxonomic Classification of the Lychee Fruit and Green Tea Plant

	Lychee Fruit	**Green Tea Plant**
Kingdom	*Plantae*	*Plantae*
Division	*Tracheophyta*	*Tracheophyta*
Class	*Magnoliopsida*	*Magnoliopsida*
Order	*Sapindales*	*Ericales*
Family	*Sapindaceae*	*Theaceae*
Genus	*Litchi Sonn.*	*Camellia L.*
Species	*Litchi chinensis* Sonn.	*Camellia sinensis* (L.) Kuntze

III.B Application of Genetic Modification (GM) Techniques

The lychee fruit and green tea leaves from which Oligonol® was derived have not been genetically modified (GM). Furthermore, no GM organisms or its derived products are used during the production of Oligonol®.

III.C Safety of the Source Organism and Foods Derived from It

Extracts of the lychee fruit and green tea leaves, both of which have an extensive history of consumption in the diet, are used as the starting materials for the manufacture of Oligonol®. The lychee fruit has a long history of cultivation in China of more than 2,000 years; it is now widely grown among subtropical regions, with China, Thailand, India, South Africa, Madagascar, Mauritius, and Australia currently being the major lychee producing countries in the world (Mitra, 2002). The European markets import approximately 20,000 tonnes of fresh lychee annually, of which nearly 50% is imported by France, and the remainder by the United Kingdom (UK) and Germany (Mitra, 2002). Although rare cases of allergic reactions to the lychee fruit has been reported (see Section X.C), the fruit is widely consumed across the world generally without any adverse effects reported.

Similarly, tea has a long history of safe consumption dating back to ancient civilisation, and it remains one of the most widely consumed beverages in the world today (Cabrera *et al.*, 2006). Both black and green teas are made from the leaves of the same plant species (*Camellia sinensis* (L.) Kuntze), though levels of polyphenols tend to be higher in green tea due to differences in the post-harvest processing (Cabrera *et al.*, 2006). Some individuals, particularly those in living Japan, have been reported to consume 10 or more cups of green tea per day (Jankun *et al.*, 1997; Fujiki *et al.*, 2002).

Preparations of green tea extracts and/or its individual components have been marketed in dietary supplement forms and promoted for its many reported health benefits. Although concerns have arisen over the use of concentrated purified forms of green tea, with case reports of hepatotoxicity reported in humans and evidence of liver and nasal toxicity observed in animals administered very high doses of green tea extracts, these findings are not expected to be relevant to the proposed uses of Oligonol®. As presented in Sections XI.B and XIII.E, the estimated intake of Oligonol® even among the highest "heavy level" consumers (*i.e.*, 1,085 mg/person/day in adults) is expected to provide approximately 174 mg/person/day of green tea catechins or more specifically, 65 mg/person/day of EGCG, which is less than the amount obtained from consumption of a 200 mL serving of green tea (see Table XI.B-1).

IV – VIII NOT APPLICABLE

IX ANTICIPATED INTAKE/EXTENT OF USE OF OLIGONOL®

Based on the SCF guidelines, the following questions must be addressed to ensure sufficient information pertaining to the intake/extent of use of the novel food:

- “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 Section IX.A.

IX.A Estimated Daily Intakes of Oligonol®

IX.A.1 Methodology

The European Food Safety Authority (EFSA) Additive and Nutritive Sources (ANS) panel has developed a food additive exposure calculation tool, the 'Food Additives Intake Model' (FAIM), to provide a first step screening tool for estimating chronic exposure to food additives. This tool allows the user to estimate the mean and high level exposure to food additives for different population groups throughout several European countries. The food consumption data within this tool are taken from the EFSA Comprehensive Database (Comprehensive Database). In 2010, the Consumption Database was built from detailed national information on food consumption. Competent Authorities in European countries provided EFSA with data on the level of food consumption by the individual consumer from the most recent national dietary survey in their country (EFSA, 2011a).

The FAIM tool allows for the estimation of chronic exposure of a given substance in food for the following population groups: toddlers, children, adolescents, adults and the elderly. To conduct the exposure assessment, food consumption data available from 26 different dietary surveys carried out in 17 different European countries are utilised. Consumption records of the Comprehensive Database were codified according to the FoodEx classification system (EFSA, 2011b). In order to perform exposure estimates, the nomenclature from the FoodEx classification system was linked to the Food Classification System (FCS) in Commission Regulation (EU) N° 1129/2011, Part D (European Commission, 2011). For the exposure calculations, mean values are calculated on all populations, by age class and survey. Calculations are performed using individual body weights. Mean intake values for the total population are calculated per age group by summing the mean exposures from all contributing food sources in a given survey. In order to calculate "heavy level" exposures, the ANS Panel assumed that an individual would be a high-level consumer of one category and would be an average consumer of the remaining food groups. Therefore, "heavy level" intakes from all contributing food sources are obtained by adding the high-level of exposure from the highest food category (calculated for consumers only) to the mean exposure values for the remaining categories (calculated for the total population). High-level values for a food category are calculated either for the 95th percentile of consumers-only, when the number of consumers is ≥60, or the mean of consumers-only when consumers <60. As an indicator of the main food categories contributing to the intakes of Oligonol®, the FAIM tool also provides all the food categories that contribute 5% or more to the estimated total exposure (given as a range of minimum-maximum percentage) for each age class. For each food category, the number of surveys for which the food category contributes 5% or more to the total mean exposure was also indicated.

IX.A.2 Anticipated Food Uses and Use-Levels

Oligonol® is proposed for use in a range of conventional foods and beverages for the general population at maximum level ranging from 100 to 1,150 mg/kg of food or mg/L of beverage. For the current exposure assessment, use levels for Oligonol® based on the maximum

proposed use level ranges per food category for use in the FAIM tool are provided in Table IX.A.2-1.

Table IX.A.2-1 Summary of the Individual Proposed Food-Uses and Maximum Use Levels for Oligonol® for Use in the EFSA FAIM Tool

Food Category Number	FCS Name Level 1	FCS Name Level 2	Proposed Maximum Use Level (mg/kg or mg/L)
1.23	Dairy products and analogues	Unflavoured fermented milk products, including natural unflavoured buttermilk (excluding sterilised buttermilk)	200
1.4		Flavoured fermented milk products including heat treated products	200
1.8		Dairy analogues, including beverage whiteners	100
5.2.1	Other confectionery including breath refreshening microsweets	Other confectionery with added sugar	1,150
5.2.2		Other confectionery without added sugar	1,150
5.3.1	Chewing gum	Chewing gum with added sugar	1,150
5.3.2		Chewing gum without added sugar	1,150
6.3	Cereals and cereal products	Breakfast cereals	800
7.2	Bakery wares	Fine bakery wares	800
12.5	Salts, spices, soups, sauces, salads and protein products	Soups and broths	410
13.3	Foods intended for particular nutritional uses as defined by Directive 2009/39/EC (European Parliament and the Council of the European Union, 2009)	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal	800
14.1.2.1	Non-alcoholic beverages	Fruit juices as defined by Council Directive 2001/112/EC (Council of the European Union, 2002)	420
14.1.4.1		Flavoured drinks with sugar	420
14.1.4.2		Flavoured drinks with sweeteners	420
14.1.5		Coffee, tea, , herbal and fruit infusions, chicory, tea; tea, herbal and fruit infusions, and chicory extracts;	420

Abbreviations: EFSA = European Food Safety Authority; FAIM = Food Additives Intakes Model; FCS = Food Classification System.

In addition to the proposed uses in conventional foods and beverages, Oligonol® is proposed for use in food supplements at levels of 200 mg/day.

IX.A.3 Estimated Daily Intakes of Oligonol® from the Proposed Food Uses

IX.A.3.1 Estimated Intakes from Conventional Foods

Using the EFSA FAIM tool, the mean intakes of Oligonol® ranged from 3.3 to14.3 mg/kg body weight/day in toddlers, 4.2 to 14.6 mg/kg body weight/day in children, 2.5 to 6.7 mg/kg body weight/day in adolescents, 1.7 to 8.8 mg/kg body weight/day in adults, and 1.4 to 7.0 mg/kg body weight/day in elderly individuals (Table IX.A.3.1-1). “Heavy level” intakes

ranged from 11.1 to 27.1 mg/kg body weight/day in toddlers, 9.1 to 25.4 mg/kg body weight/day in children, 4.2 to 13.7 mg/kg body weight/day in adolescents, 3.1 to 15.5 mg/kg body weight/day in adults, and 2.8 to 12.6 mg/kg body weight/day in elderly individuals.

Table IX.A.3.1-1 Estimated Intakes of Oligonol® from Proposed Uses in Conventional Foods Using the EFSA FAIM Tool

Population	Ages	Mean Intakes (mg/kg bw/d)		"Heavy Level" Intakes (mg/kg bw/d)	
		Lowest	Highest	Lowest	Highest
Toddlers	12 to 35 months	3.3	14.3	11.1	27.1
Children	3 to 9 years	4.2	14.6	9.1	25.4
Adolescents	10 to 17 years	2.5	6.7	4.2	13.7
Adults	18 to 64 years	1.7	8.8	3.1	15.5
Elderly	≥65 years	1.4	7.0	2.8	12.6

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

The estimated intake of Oligonol® derived using the EFSA FAIM tool was converted to a mg/day basis using reference body weight values for different age groups in the European population (EFSA, 2012a), as presented in Table IX.A.3.1-2. The mean intakes of Oligonol® ranged from 40 to 172 mg/day in toddlers, 97 to 336 mg/day in children, 108 to 288 mg/day in adolescents (10 to 14 years old), 153 to 409 mg/day in adolescents (14 to 18 years old), 119 to 616 mg/day in adults, and 98 to 490 mg/day in elderly individuals. "Heavy level" intakes ranged from 133 to 325 mg/day in toddlers, 209 to 584 mg/day in children, 181 to 589 mg/day in adolescents (10 to 14 years old), 256 to 836 mg/day in adolescents (14 to 18 years old), 217 to 1,085 mg/day in adults, and 196 to 882 mg/day in elderly individuals.

Table IX.A.3.1-2 Estimated Intakes of Oligonol® from Proposed Uses in Conventional Foods, Presented on a Milligram Per Day Basis

Population	Ages	Default Body Weights (kg)	Mean Intakes (mg/day)		"Heavy Level" Intakes (mg/day)	
			Lowest	Highest	Lowest	Highest
Toddlers	12 to 35 months	12	40	172	133	325
Children	3 to 9 years	23	97	336	209	584
Adolescents[a]	10 to 14 years	43	108	288	181	589
	14 to 18 years	61	153	409	256	836
Adults	18 to 64 years	70	119	616	217	1085
Elderly	≥65 years	70	98	490	196	882

Abbreviations: bw = body weight; d= day

[a] Separate default body weight values were provided for adolescents between the ages of 10 to 14 years of age, and for those between the ages of 14 to 18 years of age, in the EFSA guidance document (EFSA, 2012a). Therefore, the estimated intakes of Oligonol® on a mg/day basis are provided for each of these 2 age groups of adolescents.

The contributions of food groups to total exposure in each population group were examined and the top 3 food categories which contributed to total exposure (based on the maximum range of contribution) are summarised in Table IX.A.3.1-3.

Table IX.A.3.1-3 The Three Main Food Contributors to Oligonol® Intakes in Each Population Group Using the EFSA FAIM Tool

Population Group	FCS Number and Name Level 2	Minimum Range of Contribution (%)	Maximum Range of Contribution (%)	Number of Surveys ≥5% Contribution
Toddlers	14.1.2.1 - Fruit juices as defined by Council Directive 2001/112/EC (Council of the European Union, 2002)	22.7	43.2	4
	1.23 - Unflavoured fermented milk products, including natural unflavoured buttermilk (excluding sterilised buttermilk)	16.1	25.8	2
	7.2 - Fine bakery wares	12.4	25.2	3
Children	7.2 - Fine bakery wares	11.2	36.7	13
	14.1.5 - Coffee, tea, , herbal and fruit infusions, chicory, tea; tea, herbal and fruit infusions, and chicory extracts;	9.6	31.7	5
	14.1.2.1 - Fruit juices as defined by Council Directive 2001/112/EC (Council of the European Union, 2002)	8.7	56.0	14
Adolescents	14.1.4.1 - Flavoured drinks with sugar	14.8	47.1	10
	14.1.2.1 - Fruit juices as defined by Council Directive 2001/112/EC (Council of the European Union, 2002)	10.7	31.5	11
	7.2 - Fine bakery wares	9.9	33.5	11
Adults	14.1.5 - Coffee, tea, , herbal and fruit infusions, chicory, tea; tea, herbal and fruit infusions, and chicory extracts;	17.6	71.3	15
	12.5 - Soups and broths	8.2	16.6	2
	14.1.4.2 - Flavoured drinks with sweeteners	7.0	24.0	5
Elderly	14.1.5 - Coffee, tea, , herbal and fruit infusions, chicory, tea; tea, herbal and fruit infusions, and chicory extracts;	59.5	83.6	7
	12.5 - Soups and broths	15.4	15.4	1
	7.2 - Fine bakery wares	9.4	20.6	5

Abbreviations: EFSA = European Food Safety Authority; FAIM = Food Additives Intakes Model; FCS = Food Classification System.

IX.A.3-2 Estimated Exposure to Oligonol® from Food Supplements

In addition to the uses in conventional foods, Oligonol® is proposed for use in food supplements at levels of 200 mg per day. If Oligonol® food supplements are consumed at the maximum recommended amount in addition to the proposed food uses, the worst-case scenario estimate of total exposure to Oligonol® among adults, the primary population group that is expected to consume Oligonol® food supplements, is 1,285 mg/day (18.4 mg/kg body weight/day). This estimation is calculated by the addition of 200 mg/day to the highest "heavy level" intake of Oligonol® (*i.e.*, 1,085 mg/day) among adults. However, it should be noted that this is a highly conservative estimate since the intakes of Oligonol® from conventional food uses, as estimated using the FAIM tool, already represents a worst case scenario since it is assumed that very broad food categories will contain Oligonol® at the maximum use level. Moreover, it is very unlikely that individuals with the highest estimated

"heavy level" intake of Oligonol® from food uses would seek to further increase their dietary intakes by also consuming food supplements containing Oligonol®. Therefore, these intake estimates are likely to be grossly overestimated.

Furthermore, food supplements containing Oligonol® will also be subjected to certain labelling requirements, as stipulated in the food supplements legislation, which would prevent involuntary excessive dosing. Specifically, these conditions are laid down in Article 6, point 3 of *Directive 2002/46/EC on food supplements* (European Parliament and the Council of the European Union, 2002), as follows:

Without prejudice to Directive 2000/13/EC (European Parliament and the Council of the European Union, 2000), the labelling shall bear the following particulars:

1) the names of the categories of nutrients or substances that characterise the product or an indication of the nature of those nutrients or substances;
2) the portion of the product recommended for daily consumption;
3) a warning not to exceed the stated recommended daily dose;
4) a statement to the effect that food supplements should not be used as a substitute for a varied diet;
5) a statement to the effect that the products should be stored out of the reach of young children.

As such, the consumption of food supplements containing Oligonol® will be limited, and thus it is not expected to pose a safety concern.

IX.A.4 Summary

In summary, the EFSA FAIM tool was used to estimate the intakes of Oligonol® under its proposed uses in conventional foods and beverages for the European population. Overall, the highest intake level of Oligonol® on a per kg body weight basis was observed in toddlers, with the mean intakes estimated at up to 14.3 mg/kg body weight/day and "heavy level" intakes estimated at up to 27.1 mg/kg body weight/day. Using reference body weights for the European population (EFSA, 2012a), the highest intake level of Oligonol® on an absolute basis was observed in adults, with mean intakes estimated at up to 616 mg/day and the "heavy level" intakes estimated at up to 1,085 mg/day.

It should be noted that there is considerable variability in the exposure estimates derived for the proposed food uses of Oligonol®, even within individual population groups. For example, there is a 5-fold difference between the estimated mean intake level for Oligonol® in adults, with the lowest value estimated at 119 mg/day and the highest value estimated at 616 mg/day. Similarly, a 5-fold difference is observed in the "heavy level" intakes of Oligonol® for adults, with the lowest values reported at 217 mg/day and highest values reported at 1,085 mg/day. The FAIM Tool utilises data from 26 different dietary surveys carried out in 17 different European countries. As mentioned in several EFSA guidance documents

(EFSA, 2006, 2011b, 2012b), fundamental methodological differences between surveys represented in the FAIM tool account for the large range in intake estimates between countries. Methods for collecting dietary data vary from 24-hour recalls to 7-day food records. Temporal differences in dietary behaviour (the earliest survey included in the FAIM tool was conducted in 1997, and the most recent in 2009), regional/seasonal differences in dietary behaviour, and the inclusion or exclusion of region-specific foods and composite dishes (comprising a mixture of foods) into the EFSA Comprehensive Database also are expected to contribute to the large variation observed. Other general considerations regarding the use of consumption surveys also play a role in the differences in intake estimates observed. It is noted that factors influencing under-reporting or mis-reporting of food consumption, errors in composite food calculations, subject sampling error or bias, and other sources of uncertainty may differ across countries and may contribute to the broad range of intake estimates across surveys.

Additionally, the EFSA ANS Panel has noted that the exposure estimates derived using the EFSA FAIM tool should be considered as being conservative as it is assumed that all foods contain the food ingredient under consideration added at the maximum proposed use levels. It is also well-established that the length of a dietary survey affects the estimated consumption of individual users. Short-term surveys, such as the typical 2- or 3-day dietary surveys, may overestimate the consumption of food products that are consumed relatively infrequently. Therefore, it is anticipated that the actual intake of Oligonol® from the intended conditions of use will be less than estimated.

In addition to its proposed food uses in conventional foods, Oligonol® is proposed for use in food supplements at levels of 200 mg/day. It is expected that Oligonol® food supplements will be consumed primarily by adults (*i.e.*, individuals over 18 years of age). Under the worst-case scenario where Oligonol® food supplements are consumed at the maximum recommended levels by individuals with the highest estimated “heavy level” intake of Oligonol® from the proposed food uses, the total exposure to Oligonol® is estimated at 1,285 mg/day (18.4 mg/kg body weight/day). However, given that it is highly unlikely that individuals with the highest estimated “heavy level” intake of Oligonol® from food uses would seek to increase their dietary intake by also consuming Oligonol® food supplements, these estimates are likely to be grossly overestimated.

X INFORMATION FROM PREVIOUS HUMAN EXPOSURE TO OLIGONOL®

Based on the SCF guidelines, the following questions must be answered in the affirmative to ensure sufficient information pertaining to previous human exposure to the novel food:

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

- “Is there information to demonstrate that exposure to the novel food is unlikely to give rise to mitochondrial, toxicological and/or allergenicity problems?”

These questions have been addressed collectively in Sections X.A and X.C.

X.A Natural Occurrence of Polyphenols in the Diet

Polyphenols are bioactive compounds that are present in various dietary sources, including fruit, vegetables and beverages of plant origin (such as teas). Polyphenols can be divided into various classes according to their basic chemical structures; the classification of the major dietary polyphenols is summarised in Table X.A-1. Polyphenols are highly complex chemical entities; for example, flavonoids can be further divided into 6 major subclasses, which comprise over 5,000 compounds identified (Martin and Appel, 2010).

Table X.A-1 Examples of the Major Classes of Dietary Polyphenols[a]

Class	Subclass	Examples
Flavonoids	Flavan-3-ols (***main polyphenols in Oligonol***®)	Catechins (monomers)
		Proanthocyanidins (oligomers)
	Flavonols	Quercetin, myricetin
	Flavanones	Naringenin
	Flavones	Apigenin
	Isoflavones	Daidzein
	Anthocyanins	Cyanidin, peonidin
Phenolic acids	Hydroxycinnamic acids	Caffeic acid, ferulic acid, sinapic acid, *p*-coumaric acid
	Hydroxybenzoic acids	Gallic acid
Stilbenes	--	Resveratrol
Lignans	--	Secoisolaiciresinol

[a] Modified from McKay and Blumberg (2007) and Martin and Appel (2010).

Among the different classes of polyphenols, the flavonoids are one of the most common in the diet, accounting for nearly two-thirds of the total dietary intake of polyphenols (Aron and Kennedy, 2008; Song and Chun, 2008; Martin and Appel, 2010; Tsao, 2010). Specifically, flavan-3-ols and their polymeric condensation products (*i.e.*, proanthocyanidins[1]) are one of the most commonly consumed flavonoids, occurring in various dietary sources such as fruits, vegetables, plant-derived beverages (*e.g.*, tea, coffee, wine, beer), and chocolate (Santos-Buelga and Scalbert, 2000; Beecher, 2003; Gu *et al.*, 2004; Manach *et al.*, 2004; Aron and Kennedy, 2008; Thilakarathna and Rupasinghe, 2013).

A number of studies have been conducted to provide estimations of the dietary intake of polyphenols, specifically flavonoids, in various countries across the world (reviewed in Beecher, 2003; Chun *et al.*, 2007). In general, consumption of the recommended 5 servings of fruits and vegetables per day is estimated to provide a total polyphenol intake of >500 mg, of which flavonoid intake accounts for approximately 150 to 300 mg/day (Williamson and Holst, 2008; Martin and Appel, 2010). Other dietary sources such as cocoa, coffee or tea are also rich in flavonoids. For example, under typical brewing times, a 235 mL serving of tea contains between 137 to 141 mg of flavonoids (Lakenbrink *et al*., 2000). Accordingly, the total daily intakes of total polyphenols from the diet have been reported to range from less than 100 mg to more than 2,000 mg among certain individuals, depending on dietary habits

[1] Proanthocyanidins that are composed exclusively of epi(catechin) are known as procyanidins. Procyanidins are the most abundant form of proanthocyanidins in plants.

(Clifford, 2004). It has been proposed that total polyphenol intake levels of 1,000 to 1,100 mg/day can be expected among individuals who consume a balanced diet (Williamson and Holst, 2008; Martin and Appel, 2010).

X.B Regulatory Status of Oligonol® in Other Jurisdictions

In the United States (U.S.), Oligonol® is self-affirmed as Generally Recognized as Safe (GRAS) as an ingredient in conventional foods. Oligonol® is also marketed as a New Dietary Ingredient (NDI). In 2007, the U.S. Food and Drug Administration accepted an NDI notification for Oligonol® with no comments, supporting that Oligonol® is reasonably expected to be safe for use in dietary supplements at maximum recommended use levels of 200 mg/day (one 100 mg capsule taken twice daily).

X.C Allergenicity

Although Oligonol® is comprised primarily of polyphenolic compounds, a small amount of protein is detected, which accounts for approximately 1 to 2% of the final product. As such, the allergenic potential of the source materials of Oligonol® (*i.e.*, lychee fruit and green tea) are discussed herein.

Allergic reactions to the lychee fruit are thought to be rare. Several cases of anaphylactic reactions, including generalised urticaria, Quincke oedema, pruritus, bronchospasms, and dyspnoea, have been reported following ingestion of the lychee fruit (Fäh *et al.*, 1995; Giannattasio *et al.*, 1995; Niggemann *et al.*, 2002; Saraswat *et al.*, 2005; Deswarte-Antomius, 2007; Garrido *et al.*, 2007; Raap *et al.,* 2007). It has been suggested that hypersensitivity to birch pollen has been associated with allergic reaction to lychee, as well as other fruits such as apples, hazelnut, carrots and celery, due to the cross-reactivities of specific IgE antibodies (Wellhausen *et al.*, 1996). Others have also suggested that there may be possible cross-reactivity between allergens in the lychee fruit and latex (Niggemann *et al.*, 2002). Accordingly, the lychee fruit has been shown to contain significant amount of profilin, a panallergen that is present in plants that are both closely and distantly related (Fäh *et al.*, 1995; Song *et al.*, 2007). Moreover, various other allergens have been identified in the lychee fruit, including a 35 kDa protein that was shown to cross-react with birch pollen allergen (Wellhausen *et al.,* 1996; Song *et al.*, 2007). Hoppe *et al.* (2006) also identified a 28-kDa allergen present in the lychee fruit as triose-phosphate isomerase, an enzyme that has been described as an allergen in other plants (Hoppe *et al.*, 2006).

A small number of cases of occupationally-induced asthma have been reported in the literature among green tea factory workers (Shirai *et al.,* 1994*,* 1997*,* 2003). In these cases, the catechin EGCG was implicated in the IgE-mediated responses underlying the allergenic reaction (Shirai *et al.,* 1994*,* 1997*,* 2003). However, other studies have indicated that catechins from green tea may have anti-allergenic properties, with isolated catechins having inhibitory effects against type I allergic reactions (Shiozaki *et al.,* 1997; Sano *et al.,* 1999).

Overall, both lychee fruit and green tea have a long history of safe consumption, being widely consumed globally for thousands of years. Although there have been some cases of lychee fruit allergies reported in the literature, none of the participants consuming Oligonol® in the human studies conducted developed adverse effects, including symptoms of an allergic response (see Section XIII.B.4). Moreover, Oligonol® dietary supplements have been marketed in the U.S. since 2007 without any adverse events reported, including cases of anaphylactic reactions. Therefore, the potential allergenicity of Oligonol®, which is a highly purified polyphenolic product, is considered to be low.

XI NUTRITIONAL INFORMATION ON OLIGONOL®

Based on the SCF guidelines, the following question must be answered in the affirmative to ensure sufficient nutritional information pertaining to the novel food:

- “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 Sections XI.A to XI.C.

XI.A Nutritional Benefits of Oligonol®

Oligonol® is intended to serve as another source of dietary polyphenols, particularly the flavonoids such as monomeric flavan-3-ols and their polymeric condensation products (*i.e.*, procyanidins). As mentioned, flavonoid compounds are one of the most ubiquitous polyphenols consumed in the diet from plant sources (Aron and Kennedy, 2008; Song and Chun, 2008; Tsao, 2010). High intake of foods that are rich in polyphenols (*e.g.*, fruits, vegetables and whole grains) have been associated with lowered risk of many chronic diseases, including cardiovascular disease, cancer, neurodegeneration, and chronic inflammation (Aron and Kennedy, 2008; Martin and Appel, 2010; Tsao, 2010). The beneficial health effects of polyphenols have been mainly attributed to their potent antioxidant activity (Martin and Appel, 2010; Tsao, 2010). There is a wealth of evidence demonstrating that polyphenols can act as direct radical scavengers of numerous reactive oxygen species (ROS), as well as suppress the generation of free radicals (Martin and Appel, 2010; Tsao, 2010).

XI.B Nutritional Equivalence to Existing Foods

Oligonol® is intended for use as an ingredient in foods to supplement the levels of polyphenols that are already consumed as part of a normal diet. As discussed above in Section X.A, the monomeric flavan-3-ols and oligomeric procyanidins present in Oligonol® are consumed naturally through various plant-derived sources in the diet. One of the major dietary source of monomeric-flavan-3-ols is green tea; the estimated intake of the monomeric flavan-3-ols even among the highest "heavy level" consumers of Oligonol® is less than the amount that would be obtained from drinking 1 cup of green tea (see Table XI.B-1). Similarly, the estimated intake of procyanidins even among the highest "heavy level" consumers of Oligonol® is comparable to the amount that would be consumed from several servings of fruit that is rich in procyanidins (Table XI.B-2). It should be noted again that the exposure estimates represent the worst-case scenario, and the estimated intakes derived using the EFSA FAIM Tool were found to vary by more than 3- to 5-fold depending on the dietary surveys used across the 17 different European countries.

Table XI.B-1 Intake of Monomeric Flavan-3-ols from Consumption of Oligonol® Compared to Drinking Green Tea

	Oligonol®			Green tea
	Composition (%)	Adults		Intake from 200 mL serving[b] (mg)
		Highest Mean Intake[a] (mg/day)	Highest Heavy Level Intake[a] (mg/day)	
(+)-Catechin and (-)-EC	8	49.3	86.8	21.6
(-)-EGC	--	--	--	33.4
(-)-ECG	2	12.3	21.7	39.5
(-)-EGCG	6	37.0	65.1	155.6
Total monomeric flavan–3–ol content	16	98.6	173.6	250.1

Abbreviations: (-)-EC = (-)-Epicatechin; (-)-EGC = (-)-Epigallocatechin; (-)-ECG = (-)-Epicatechin-3-gallate; (-)-EGCG = (-)-Epigallocatechin gallate

[a] Intake of monomeric flavan-3-ols was calculated for adults, the population group with the highest estimated intake of Oligonol® from its proposed uses in conventional foods, as derived using the EFSA FAIM tool (see Table IX.A.3.1-2). The highest mean intake of Oligonol® was estimated at 616 mg/day, and the highest "heavy level" intake was estimated at 1,085 mg/day.

[b] Calculated based on data taken from USDA (2007).

Table XI.B-2 Intake of Procyanidins from Consumption of Oligonol® Compared to Selected Dietary Sources[a]

	Type of Interflavan Linkages	Total Procyanidin Content (mg/100 g food)[b]	Serving Size (g)	Estimated Intake Per Serving (mg/day)	
Choke berry	B	663.7	80	531.0	
Cranberry	A, B	418.8	80	335.0	
Blueberry	B	255.8	80	204.6	
Plum	A, B	215.9	80	172.7	
Apple	B	104.4	80	83.5	
Peach	B	67.3	80	53.8	
Green Pear	B	42.3	80	33.8	
Peanut	A, B	15.6	25	3.9	
Cocoa[c]	B	1635.9	--	--	
Sorghum, Sumac[c]	B	1919.5	--	--	
	Type of Interflavan Linkages	**Total Procyanidin Content (%w/w)**	**Serving Size (g)**	**Estimated Daily Intake (mg/day)[d]**	
				Mean Intake	**"Heavy Level" Intake**
Oligonol®	A, B	70	--	431	760

[a] Estimated daily consumption was calculated using the data on proanthocyanidin content in foods that was published in Prior and Gu (2005), and the typical serving sizes that was published by Lewis *et al.* (2012). The foods selected are rich in procyanidins, and includes those that contain both the A-type and B-type linkages (*e.g.*, cranberries, plums, and peanuts), similar to the procyanidins present in Oligonol®. Foods that contain other forms of proanthocyanidins in addition to procyanidins are not included here.

[b] On wet weight basis.

[c] Procyanidin content was reported for unprocessed cocoa and sumac sorghum (Gu *et al.*, 2004); therefore, the estimated daily amount consumed is not calculated.

[d] Intake of procyanidins was calculated for adults, the population group with the highest estimated intake of Oligonol® from its proposed uses in conventional foods, as derived using the EFSA FAIM tool (see Table IX.A.3.1-2). The highest mean intake of Oligonol® was estimated at 616 mg/day, and the highest "heavy level" intake was estimated at 1,085 mg/day.

XI.C Other Nutritional Considerations

The absorption, metabolism, distribution, and excretion of the polyphenolic constituents in Oligonol® are discussed below in Section XIII.A. As mentioned, polyphenols are widely consumed in the diet, and the intake of polyphenols from the intended uses of Oligonol® in foods and food supplements are not expected to produce adverse nutritional effects.

XII MICROBIOLOGICAL INFORMATION ON OLIGONOL®

Based on the SCF guidelines, the following question must be addressed to ensure sufficient microbiological information on the novel food:

- “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 Presence of Microorganisms

Analytical data on the levels of microorganisms in 3 non-consecutive batches of Oligonol® are presented in Table I.C-1 in Section I above. The results confirm that the levels of microbiological contamination are either below detection limits or well below the specification limits.

XIII TOXICOLOGICAL INFORMATION ON OLIGONOL®

Based on the SCF guidelines, the following questions must be addressed to ensure sufficient toxicological information pertaining to the novel food:

- “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 through XIII.F.

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

As mentioned, Oligonol® is composed primarily of monomeric flavan-3-ols and low molecular weight oligomeric procyanidins derived from lychee fruit (*L. chinensis* Sonn.) and green tea leaves [*C. sinensis* (L.) Kuntze]. As such, the metabolic fate of Oligonol® can be extrapolated from studies conducted with its monomeric and oligomeric constituents (*i.e.*, monomeric flavan-3-ols and oligomeric procyanidins). A large number of pre-clinical and clinical studies have been conducted to investigate the metabolic fate of the polyphenol constituents in Oligonol®; an overview of the ADME processes of polyphenols is provided below in Section XIII.A.1. Additionally, a human bioavailability study has been conducted using the Oligonol®-like product (Fujii *et al.*, 2007). This Oligonol®-like product was manufactured using a similar oligomerisation process as Oligonol®, but using different starting materials (*i.e.*, fruits rich in proanthocyanidins such as grape seed extracts, apples, and persimmons). Similar to Oligonol®, the Oligonol®-like product is composed of 15 to 20% monomers, 8 to 12% dimers, and 5 to 10% trimers. The results of this study are summarised in Section XIII.A.2.

XIII.A.1 Polyphenol Constituents of Oligonol®

XIII.A.1.1 Monomeric Flavan-3-ols

Although the monomeric flavan-3-ols are present in diverse food sources, the majority of the bioavailability studies have been conducted using cocoa or tea as the test article. In general, monomeric flavan-3-ols are absorbed to a greater extent than dimers, trimers, and larger oligomers (Manach *et al.*, 2005; Yang *et al.,* 2008). Unlike other flavonoids, flavan-3-ols are not glycosylated, and therefore they do not require hydrolysis in the gastrointestinal tract prior to absorption (Manach *et al.*, 2004; Yashin *et al.*, 2012).

(-)-Epicatechin, (-)-epigallocatechin (EGC), ECG, and EGCG have been reported to be absorbed from the gastrointestinal tract following oral administration in mice and rats (Okushio *et al.*, 1995, 1996; Nakagawa and Miyazawa, 1997; Suganuma *et al.*, 1998; Donovan *et al.*, 2001). Both the intact form and metabolites of these monomeric flavan-3-ols have been identified in the plasma of rodents following oral administration (Piskula and Terao, 1998; Harada *et al.*, 1999; Kim *et al.*, 2000). Studies conducted in humans suggest that the bioavailability of catechins (*i.e.*, epicatechin, EGC, ECG, and EGCG) is generally low; Manach *et al*. (2005) used data from 97 human studies that investigated the kinetics and extent of polyphenol absorption, and determined the mean relative urinary excretion (as % of intake) to be 18.5% for (epi)catechin (range: 2.1 to 55.0%), 11.1% for EGC (range: 4.2 to 15.6%), and 0.06% for EGCG (range: 0.0 to 0.1%). In these studies, relative urinary excretion was used as an indicator of absorption though it is possible that underestimation may have occurred for compounds (such as EGCG) that are highly excreted in the bile (Manach *et al.*, 2005). There is some evidence from both animal and human studies to suggest that the bioavailability of catechins may be enhanced if they are consumed under fasting compared to fed states (Chow *et al.*, 2005; Isbrucker *et al.*, 2006; Kapetanovic *et al.*, 2009).

Following absorption, monomeric flavan-3-ols predominantly undergo methylation, glucuronidation, and/or sulfation (Yang *et al.*, 2008; Yashin *et al.*, 2012). These conjugated metabolites have been detected in the plasma and urine of animals and humans. For example, the major circulating metabolites of epicatechin has been identified as epicatechin-3'-O-glucuronide, 4'-O-methylepicatechin-3'-O-glucuronide, 4'-O-methylepicatechin-5- or 7-glucuronide, and the aglycones epicatechin and 4'- methylepicatechin (Manach *et al.*, 2005). Although catechins occur mostly as conjugated forms in systemic circulation, EGCG is a notable exception in that a large proportion (77 to 90%) has been detected in the plasma as the free form (Manach *et al.*, 2005; Yashin *et al.*, 2012).

Monomeric flavan-3-ols that are not absorbed in the upper gastrointestinal tract may be subjected to metabolism by the colonic microflora in the lower gastrointestinal tract into metabolites that may then be subsequently absorbed (reviewed in Manach *et al.*, 2005). Accordingly, the conjugated forms of the microbial metabolites of catechins [*e.g.*, 5-(3',4',5'-trihydroxyphenyl)valerolactone, 5-(3',4'-dihydroxyphenyl)valerolactone, and 5-(3',5'-dihydroxyphenyl)valerolactone] have been detected in the plasma and urine of human volunteers following ingestion of green tea (Meng *et al.*, 2002). These metabolites were present at levels that were 8 to 25 times higher than those of the unchanged compounds, and accounted for 6 to 38% of the ingested dose of EGC and epicatechin (Li *et al.*, 2000). Overall, catechins and their metabolites are known to be rapidly eliminated through the urinary, faecal, and biliary routes (Manach *et al.*, 2005).

XIII.A.1.2 Proanthocyanidins

Among the flavonoids, the proanthocyanidins are the most poorly absorbed, with absorption being 10- to 100-fold lower than their monomeric constituents in both preclinical and clinical studies (reviewed in Manach and Donovan, 2004; Manach *et al.*, 2005; Aron and Kennedy, 2008). Although *in vitro* studies suggests that oligomeric procyanidins could be hydrolysed to smaller monomeric and dimeric units under conditions simulating those in the stomach (Kuhnau, 1976; Spencer *et al.*, 2000), this finding has not been supported by studies conducted in animals or humans (Donovan *et al.*, 2002; Rios *et al.*, 2002). As such, the majority of ingested proanthocyanidins are expected to escape gastric degradation and absorption in the small intestines, but instead, become metabolised by the colonic microflora in the lower gastrointestinal tract. *In vitro* incubation of purified, radiolabelled proanthocyanidin oligomers with isolated human colonic microflora resulted in the production of various phenolic acids, including *m*-hydroxyphenylpropionic acid, *m*-hydroxyphenylacetic acid, and their *p*-hydroxy isomers, *m*-hydroxyphenylvaleric acid, phenylpropionic acid, phenylacetic acid, and benzoic acid (Déprez *et al.*, 2000). Some of these metabolites have also been detected in the urine of humans fed chocolate (which are rich in procyanidins) (Rios *et al.*, 2003), as well as animals that were orally administered purified catechins, procyanidin B3 (dimer), procyanidin C2 (trimer), and larger oligomeric procyanidins (Gonthier *et al*., 2003).

XIII.A.2 Polyphenols from Oligonol®-like Product

In a bioavailability study, 30 subjects (23 to 62 years of age) were randomly divided into 3 groups (5/sex/group) and given capsules containing 200 mg grape seed extract (control), 100 mg of an Oligonol®-like product, or 200 mg of the Oligonol®-like product, to be taken orally for a period of 92 days (Fujii *et al*., 2007). Blood samples were collected at 0, 2, 4, and 6 hours after dosing, as well as on Days 28 and 92, and polyphenol concentrations in the serum were measured. A peak in total polyphenol concentration in the serum was observed at 2 hours following ingestion of the Oligonol®-like product (at 200 mg/day dose), whereas no clear peak was observed in the group consuming the control grape seed extract product. Furthermore, the steady-state levels of polyphenols in the serum at Day 92 were dose-dependently higher in subjects administered the Oligonol®-like product. Compared to controls, the serum levels of polyphenols being 4 and 10 times higher in subjects receiving the 100 and 200 mg/day dose of the Oligonol®-like product, respectively. Overall, this study demonstrates that the polyphenols in the Oligonol®-like product are bioavailable, and that the continuous intake of an Oligonol®-like product results in elevated levels of polyphenols in the serum.

XIII.A.3 Summary

Given that Oligonol® consists mainly of monomeric flavan-3-ols and procyanidins, it is expected to undergo similar ADME processes as naturally occurring polyphenolic compounds. In general, the flavan-3-ol monomers are absorbed to a greater extent than its oligomeric forms, with the absorption of procyanidins reported at 10 to 100-fold less than the monomers (Manach and Donovan, 2004; Manach *et al.*, 2005; Aron and Kennedy, 2008; Yang *et al.*, 2008). Following absorption, the majority of monomeric flavan-3-ol, with the exception of EGCG, becomes conjugated *via* methylation, glucuronidation, and/or sulfation (Yang *et al.*, 2008; Yashin *et al.*, 2012). The monomeric flavan-3-ols, as well as the oligomeric procyanidins, that escapes absorption and passes into the lower intestinal tract intact may be metabolised by the colonic microflora into metabolites (*e.g.*, phenylvalerolactones and phenolic acids) that can be subsequently absorbed. Similar to other polyphenols, flavan-3-ols and their metabolites are rapidly excreted through the urine, faeces, and bile (Manach *et al*., 2005). Additionally, one human study conducted with an Oligonol®-like product that is similar to Oligonol® with respect to the manufacturing process and composition indicate that polyphenol levels in the serum are elevated following consumption of the Oligonol®-like product at doses of 200 mg/day (Fujii *et al.*, 2007).

XIII.B Toxicological Studies Conducted with Oligonol®

XIII.B.1 Acute Toxicity

Sprague-Dawley rats (10/sex/group) were administered a single dose of 2,000 mg/kg body weight of Oligonol® in water by gavage, and the vehicle (water) was administered to control animals (Fujii *et al.,* 2008). All animals were observed for signs of toxicity, including mortality and moribundity, for 14 days. At the end of the 14-day observation period, all animals were

terminated and the organs were removed and examined macroscopically. There were no deaths during the study period, and the body weight of animals administered Oligonol® did not differ significantly from animals in the control groups. In 4 females administered Oligonol®, mucous in the faeces was observed within the first day, and salivation was observed in 1 female at 30 minutes after administration. These findings were not reported in any of the males. No abnormalities were revealed upon macroscopic examination. Based on the results of this study, the authors concluded the oral median lethal dose (LD_{50}) for Oligonol® to be greater than 2,000 mg/kg body weight in male and female rats.

XIII.B.2 Subchronic Toxicity

Oligonol® has been administered to rats by gavage in two conventional 90-day oral toxicity studies, and one 90-day oral toxicity study has been conducted where Oligonol® was added to the diet of mice. The results of these studies are summarised below.

XIII.B.2.1 Rats

Gavage Study #1

In a subchronic oral toxicity study conducted according to Good Laboratory Practice (GLP), groups of 6 male and 6 female Sprague-Dawley rats (3 weeks of age) were administered 0 (control), 100, 300, or 1,000 mg/kg body weight Oligonol® by gavage once daily for a period of 90 days (Fujii *et al.,* 2008). Rats were acclimatised for 2 weeks prior to initiation of dosing and were provided with a commercial pelleted diet and tap water *ad libitum*. Animals were observed for general appearance twice daily, and food consumption and body weights were measured twice weekly. During Week 13, urine samples were collected immediately after dosing from non-fasted animals using metabolic cages. Urine collected during the first 3 hours was measured for pH, protein, glucose, ketone bodies, urobilinogen, bilirubin, and occult blood. The accumulated urine samples (collected for 21 hours) were measured for urine volume and specific gravity. On the day of necropsy, blood samples were collected and analyzed for red blood cell count, haematocrit, haemoglobin concentration, mean corpuscular haemoglobin concentration, platelet count, white blood cell count, reticulocyte count, and differential white blood cell count, and prothrombin time and activated partial thromboplastin time were measured in the plasma. Clinical chemistry parameters were measured including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), *gamma*-glutamyl transpeptidase (GGPT), and glucose. Serum from blood samples was analyzed for total cholesterol, triglycerides, total bilirubin, urea nitrogen, creatinine, calcium, inorganic phosphorus, total protein, sodium, potassium, chloride, protein fraction, and albumin/globulin ratio. On Day 90, all animals were sacrificed and organs and tissues were examined macroscopically. The brain, pituitary gland, thyroid, thymus, adrenal glands, spleen, heart, liver, kidneys, testes, epididymides, and ovaries were weighed, and relative organ weights were calculated. In addition, all gross lesions, lung, mesenteric lymph node, pancreas, tongue, manidibular lymph node, salivary glands, mammary gland, skin, eyeballs, Harderian glands, sternum, right femur bone, spinal cord, skeletal muscle, thoracic aorta, trachea, oesophagus, stomach, duodenum, jejunum, ileum,

caecum, colon, rectum, urinary bladder, prostate, seminal vesicle, uterus, vagina, and peripheral nerve were resected with a border of normal tissue. Tissue samples from all animals were histopathologically examined.

There were no significant differences in body weight or food consumption between the control group and the treated groups throughout the 90-day period. There were no abnormal findings in general appearance or clinical observations for animals in the 100 and 300 mg/kg body weight dose groups in either sex. However, in the 1,000 mg/kg body weight group, bloody faeces was observed in 1 male on Days 86, 87, 90, and on the day of necropsy. Urinalysis parameters were within normal ranges for all animals with the exception of an increase in urine volume in 2 female animals in the 100 mg/kg body weight/day dose group. The authors suggested that the high values may have resulted from contamination of the urine samples with the drinking water bottles. In the 1,000 mg/kg body weight/day group the urine was orange-yellow in males and females.

There were no treatment related effects observed in clinical chemistry parameters. A significant increase in activated partial thromboplastin time in the males of the 1,000 mg/kg body weight/day dose group occurred. This result was not observed in the female rats, and there was no dose-response relationship observed in males. In female rats, the reticulocyte count was significantly decreased in the 100 and 1,000 mg/kg body weight/day dose groups when compared to the control group. This was not observed in the male rats at any doses or in females in the 300 mg/kg body weight/day dose group. All other haematological parameters in animals receiving Oligonol® did not differ significantly from controls. The blood biochemistry parameters were mostly normal with the exception of a significant decrease in urea nitrogen in males in the 1,000 mg/kg body weight/day dose group compared to the control group. This effect was not noted in females, and was not considered to be test-article related. In females at the 1,000 mg/kg body weight/day dose group, total protein and triglycerides were significantly decreased compared to the control group. This effect was not considered to be treatment related because it was not seen in males and did not follow a dose-response relationship.

There were no abnormal gross necropsy findings in either sex at the 100 mg/kg body weight/day dose group. One male in the 300 mg/kg body weight/day dose group was observed to have gray discoloration of the mucosa of duodenum and focal red discoloration of mucosa of the jejunum. In the 1,000 mg/kg body weight/day dose group, all males and females had gray discoloration of the mucosa of the duodenum. Males in the 300 mg/kg body weight/day dose group had significantly lower absolute thymus weights than the control animals, however, there were no effects observed in the 1,000 mg/kg male groups or in the females. Females in the 1,000 mg/kg body weight/day dose group had significantly lower absolute ovary weights than the control group. However, the relative weight was not significantly different from the control and no histological changes were observed, and so, these finding were not considered to be toxicologically significant. There were no significant histopathological findings observed in any of the tissues examined with the exception of a dose-dependent deposition of brown pigment in the lamina propria of the duodenum following staining. Slight deposition was observed in all rats in the 300 mg/kg body

weight/day dose group and the intensity increased to moderate in rats in the 1,000 mg/kg body weight/day dose group. The pigment was not observed in any other tissues in the gastrointestinal tract and was not accompanied by an inflammation or changes in the tissue. Although this finding was considered to be treatment related, it was not considered to be toxicologically significant as there was no change in the tissues or inflammation. The authors suggested that the brown pigment was probably an accumulation of oxidised phenolics, resulting in the positive staining. The authors considered the no-observed-adverse-effect level (NOAEL) to be 1,000 mg/kg body weight/day, the highest dose tested.

Gavage Study #2

In an unpublished study conducted under GLP and in accordance with the Organisation for Economic Co-operation and Development (OECD) Guidelines, CD-rats (10/sex/group) were administered Oligonol® at 0, 100, 300, or 800 mg/kg body weight/day by gavage for 90 days (Leuschner, 2011). A separate set of animals in the control and high-dose group (5/sex/group) underwent an additional 6-week recovery period following the 90-day treatment period. Rats were acclimatised for 7 days prior to initiation of dosing, and commercial pelleted diet and tap water were available *ad libitum*. Animals were observed for general appearance at least once daily, while detailed clinical observations, food consumption and body weights were measured weekly. A neurological screening test was conducted at study termination (Week 13) or at the end of the recovery period (Week 19), and included sensory reactivity to various types of stimuli, as well as assessment of grip strength and motor activity. Blood samples were collected following an overnight fast at study termination or at the end of the recovery period for determination of haematological and clinical biochemistry parameters. Urine samples were collected following overnight fast at the study termination or at the end of the recovery period for urinalysis. Ophthalmological examination was performed prior to the start of the study, at study termination, and at the end of the recovery period. Necropsy was performed at study termination, and organs/tissues were examined for macroscopic and histopathological changes.

No treatment-related changes were noted in mortality, clinical signs (*i.e.,* behaviour, external appearance, or faeces), or functional observation tests throughout the duration of the study. No treatment-related effects were found during the neurological screen test (*i.e.*, fore- and hind limb grip strength, spontaneous motility) and ophthalmological examination at Week 13 or 19. The body weight of male animals treated with 800 mg/kg body weight/day of Oligonol® was approximately 9% less than the body weight of control animals at Week 2 of treatment ($p \leq 0.01$). However, no other significant changes in body weight were reported over the course of the study. No treatment-related changes in food or water intake were reported by the study authors.

Haematological parameters (*i.e.,* haemoglobin, red blood cell count, white blood cell count, reticulocytes, platelet count, differential blood count, haematocrit, thromboplastin time, activated partial thromboplastin time, mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration) did not vary by treatment. A significant increase in plasma total bilirubin (by 26%) was observed at the end of the study

in females treated with 800 mg/kg body weight/day dose compared to controls, although levels returned to those observed in controls upon cessation of treatment with Oligonol® at the end of the 6-week recovery period. No other treatment-related changes in clinical biochemistry parameters (*i.e.*, albumin, cholesterol, creatinine, glucose, total protein, triglycerides, blood urea, ALT, AST, ALP, lactate dehydrogenase, and electrolytes) were reported. Specific gravity of the urine was increased significantly by 2% ($p \leq 0.01$) at the end of the study in females treated with 800 mg/kg body weight/day of Oligonol® compared to controls. This effect subsided upon cessation of Oligonol® treatment at the end of the recovery period. No other treatment-related changes in urinalysis parameters (*i.e.*, urine pH, urine volume, analyte concentrations of nitrite, protein, glucose, ketones, urobilinogen, bilirubin and haemoglobin, urine colour and microscopically analyzed urine sediments) were observed.

Macroscopic examination at necropsy did not reveal any treatment-related abnormalities. A small number of macroscopic changes were noted in the lungs, liver, thymus, stomach, adrenals and ovary. However, these changes were considered to be spontaneous and within the normal range of variation as they were not dose-dependent and occurred in animals in the control group. No treatment-related changes in the relative and absolute organ weights were found, and histopathological examination did not reveal any treatment-related changes. Examination of the testicles in males treated with 800 mg/kg body weight/day of Oligonol® revealed normal spermiogenesis, sperm count, and no evidence of degenerating spermatocytes and spermatids. Based on these results, the authors concluded the no-observed-effect level (NOEL) of Oligonol® to be 300 mg/kg of Oligonol®, and the NOAEL to be 800 mg/kg.

XIII.B.2.2 Mice

In a non-GLP study, the safety of Oligonol® and lychee fruit extract (a starting material in Oligonol®) were assessed in ddY male mice (Fujii *et al.,* 2008). The mice were 5-weeks old and acclimatised for 1 week before the start of the study. Groups of 5 mice were fed CE-2 feed powder for rodents diet supplemented with 0 (control), 200 mg lychee fruit extract/kg body weight, 2 mg Oligonol®/kg body weight, 20 mg Oligonol®/kg body weight, or 200 mg Oligonol®/kg body weight for a 90-day period. Animals were housed 5 per cage in polycarbonate cages. Body weights and food consumption were measured twice weekly, and diets were prepared daily to provide the exact dosage. General status of the animals was recorded twice weekly. On Day 90, all mice were terminated, blood samples were collected for analysis of biochemical parameters, and organs including brain, heart, liver, kidney, and spleen were removed for macroscopic examination and weighing. Serum was obtained and glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), glucose, blood urea nitrogen, creatinine, triglyceride, total cholesterol, total protein, albumin, and albumin/glucose ratio were measured. All animals survived until the end of the study period and there were no adverse effects observed. Body weights were not significantly different between mice fed lychee fruit extract or Oligonol® compared with mice fed the control diet. Similarly, there were no significant differences in absolute and relative

organ weights between the groups. Analysis of biochemical parameters did not reveal any abnormalities and the lack of toxicity in this study supports the findings in the rat studies.

XIII.B.3 Mutagenicity and Genotoxicity

Fujii *et al.* (2008) conducted an *in vivo* micronucleus assay, a bacterial reverse mutation test, and an *in vitro* chromosomal aberration assay to assess the mutagenic/genotoxic potential of Oligonol®. In the micronucleus assay, 7-week-old male SPF mice were randomly assigned to 5 groups of 6 animals, representing a control group, a positive control group, and 3 treatment groups (500, 1,000, or 2,000 mg/kg body weight). A commercial pelleted diet and tap water were available *ad libitum* for all animals throughout the study period. Mitomycin C was the positive control and administered at a dose of 1 mg/kg body weight, and carboxymethylcellulose sodium solution was used as the negative control, as it was the vehicle for Oligonol®. With the exception of the positive controls treatments conducted twice by gavage at a 24-hour interval, each animal was observed for general appearance before and after the first and second administration. Animals were weighed before the first administration and 24 hours after the final administration. All animals were killed 24 hours after the final administration of the control or test substances and bone marrow smears were prepared for each animal following the extraction of marrow from both femurs. The ratio of polychromatic to normochromatic erythrocytes, the incidence of micronuclei, and the percent of polychromatic erythrocytes were measured. The general appearance of the mice in all groups was normal before and after administration, and body weights were not significantly different between groups. There were no statistically significant differences between the control and Oligonol® treated groups in the micronuclei counts or in the percent of polychromatic erythrocytes. The percent of polychromatic erythrocytes to total erythrocytes was normal, indicating there was no suppression of bone marrow function. The positive control group had a significant increase in micronuclei compared to the control group, which validated the test system. Based on the results of this study, it can be concluded that Oligonol® is not clastogenic *in vivo.*

A bacterial reverse mutation test also was conducted with Oligonol® using *Salmonella typhimurium* strains TA100, TA1535, TA98, TA1537, and *Escherichia coli* WP2*uvr*A, with and without metabolic activation. Distilled water was used as the negative control and commonly accepted mutagens [*i.e.*, 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide, sodium azide; and 9-Aminoacridine hydrochloride hydrate] were used as the positive control. An initial concentration range finding test was carried out with doses from 1.5 to 5,000 µg/plate, and from those results, 156, 313, 635, 1,250, 2,500, and 5,000 µg/plate were selected as the doses to be used for the test. In the concentration range finding test and the main test the average number of revertant colonies in the Oligonol® treated groups was less than twice that of the negative control group, and no concentration-dependent increases in the number of revertant colonies were observed either without or with metabolic activation. Therefore, the findings from both the range-finding test and the main test demonstrate that Oligonol® is not mutagenic in the bacterial strains tested.

A chromosome aberration test was conducted with Oligonol® using cultured Chinese hamster lung cells. Mitomycin C was used as the positive control without metabolic activation at concentrations of 0.1 and 0.05 µg/mL in the 6- and 24-hour treatments, respectively. Benzo[a]pyrene was used as the positive control substance in the assays with metabolic activation. Incubation with the test substance with and without metabolic activation for 6 hours, and without metabolic activation for 24 hours was followed as the protocol for the preliminary test. In the main test, Oligonol® concentrations were used at 19.5, 39.1, 78.1, 156, 313, 469, and 625 µg/mL for the 6-hour test with metabolic activation, 9.8, 19.5, 39.1, 78.1, 104, 130, and 156 µg/mL for the 6-hour test without metabolic activation, and at 4.9, 9.8, 19.5, 29.3, 39.1, 58.6, 78.1 µg/mL for the 24-hour treatment without metabolic activation.

In the 6-hour preliminary test, inhibition of cell growth was observed at 156 µg/mL and above without metabolic activation, and at 635 µg/mL and above with metabolic activation. In the 24-hour test, inhibition of cell growth was observed at a dose level of 78.1 µg/mL. In the main test, after 6 hours of incubation, inhibition of cell growth was seen at 130 µg/mL and above without metabolic activation, and at 469 µg/mL and above with metabolic activation. Following 24 hours of incubation, inhibition of growth was observed at 39.1 µg/mL and above. There was no precipitation of the test substance and there were no effects on the pH of the culture solution in any test series. In both the 6- and 24-hour treatment groups, the incidences of structural aberrations were less than 5% at all concentrations tested. In both the 6- and 24-hour test groups, with or without metabolic activation the incidence of numerical aberration (polyploidy) of chromosomes increased in a concentration-dependent manner. The authors concluded that Oligonol® caused numerical, but not structural aberrations under the conditions tested. The increased polyploidy observed was considered to be a non-genotoxic event.

Together, the results of these micronucleus, reverse mutation, and chromosome aberration tests provide evidence that Oligonol® is not genotoxic or mutagenic.

XIII.C Human Studies Conducted with Oligonol®

Three unpublished human studies have been conducted with Oligonol®. In the first study, 6 healthy volunteers (5 male and 1 female) consumed 200 mg Oligonol® twice a day (400 mg/day) for 3 months. Blood biochemistry parameters were evaluated on Days 0, 30, and 92, and included indicators of liver and kidney function such as GOT, GPT, GGPT, blood urea nitrogen (BUN) and creatinine. No changes occurred over the treatment period and no significant adverse events were reported. One volunteer experienced transient diarrhoea for the first week that resolved without treatment.

In another study, Oligonol® was administered at a daily dose of 300 mg twice a day (600 mg/day) for 14 days to 29 healthy subjects (16 females and 13 males). Parameters evaluated included haematology (white blood cells, haemoglobin, haematocrit, and platelets) and serum biochemistry (calcium, creatinine, total protein, albumin, globulin, bilirubin, BUN, glucose, AST, ALT, ALP, Na, K, Cl, CO_2), serum malondialdehyde as a measure of lipid

peroxidation, and electrocardiogram (ECG) readings. There were no changes in any parameter between baseline and the final visit. Twenty-one subjects (72.4%) reported no adverse symptoms during the trial at the interim or final assessment. Three subjects (10.3%) had abdominal discomfort and bloating at both interim and final visits and 5 subjects (17.3%) reported other minor transient symptoms including dry mouth, increased appetite, fatigue and headache, mild nausea, and loose stools at either the interim or the final visit.

A double-blind, randomised, placebo-controlled study was conducted in 76 male and female subjects with pre-hypertension or hypertension (Stage 1 or Stage 2) to determine the efficacy of Oligonol® in reducing blood pressure. Twenty-three subjects were assigned to receive placebo, while 28 and 25 subjects received 100 and 200 mg/day of Oligonol®, respectively, for 60 days. Fasting blood samples were collected at baseline and at the end of the study (Day 60). Safety-related endpoints examined included haematology (*i.e.*, WBC count with differential, haemoglobin, haematocrit, platelet count) and serum biochemistry parameters (*i.e.*, glucose, total bilirubin, total protein, ALP, ALT, AST, BUN, creatinine, calcium, sodium, potassium, chloride, bicarbonate, lipid profile). No adverse events were reported in subjects treated with Oligonol®, and no treatment-related changes in haematology and serum biochemistry parameters were observed. Subjects treated with Oligonol® at both doses had fasting mean blood glucose levels greater than 100 mg/dL following the 60 days of treatment (106.1±3.9 mg/dL for 100 mg/day dose and 112.1±49.0 mg/dL for 200 mg/day dose); however, this finding was also observed during the screening visit, and may be driven by individuals who did not comply to the overnight fast prior to blood sample collection.

Overall, the data from these clinical studies provide additional evidence to support the safety of Oligonol®. No adverse side effects or treatment-related changes in haematology and serum biochemistry parameters were reported when Oligonol® was orally administered at doses up to 400 mg/day for 3 months, 600 mg/day for 14 days, or 200 mg/day for 60 days.

XIII.D Safety of an Oligonol®-like Product

As mentioned in Section IV.B.2, Amino Up also manufactures an Oligonol®-like product using the same oligomerisation process as Oligonol®, but with different starting materials (*i.e.*, fruits rich in proanthocyanidins such as grape seed extracts, apples, and persimmons). Similar to Oligonol®, the Oligonol®-like product is composed of 15 to 20% flavan-3-ol monomers, 8 to 12% dimers, and 5 to 10% trimers. Based upon the similarity in composition, the data from toxicity studies (*i.e.*, acute and short-term) and human clinical studies conducted with the Oligonol®-like product can be used to corroborate the safety of Oligonol®.

XIII.D.1 Acute Toxicity

The acute oral toxicity of an Oligonol-like product was assessed in single dose studies in rats and mice (Fujii *et al.,* 2007). Five-week-old male and female Sprague-Dawley rats (5/sex/group) were administered 2,000 mg/kg body weight of the Oligonol-like product by

gavage. No mortalities were observed and the LD_{50} was determined to be >2,000 mg/kg body weight. In another study, 8-week-old male and female ddY mice were administered a single dose of Oligonol-like product at 2,500, 5,000, 7,500, and 10,000 mg/kg body weight by gavage. Mortality was observed in 1/8, 2/7, 3/3, and 17/18 mice in the 2,500, 5,000, 7,500, and 10,000 mg/kg body weight dose, respectively, and the LD_{50} was determined to be 5,000 mg/kg body weight.

XIII.D.2 Short-Term Toxicity

In a 1-month study conducted by Fujii *et al.* (2007), 7-week-old ddY mice were randomly allocated to 4 groups of 6 mice each and fed diets supplemented with either grape seed polyphenols at 200 mg/kg body weight/day, or an Oligonol-like product at 3.33, 24.6, or 200 mg/kg body weight/day. A control group fed a basal diet also was included. Food consumption and body weights were measured every 2 days and at the end of the 4-week study period all mice were terminated and serum was collected for the measurement of biochemical parameters including: glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, triglyceride, total cholesterol, BUN, total protein, albumin, and albumin/globulin. There were no visible signs of adverse effects or toxicity and there was no significant changes in body weight gain or food consumption between the groups. Serum biochemistry parameters were all within normal limits and did not reveal any abnormalities. The authors concluded that the Oligonol-like product administered at a level of 200 mg/kg body weight/day, for a 1-month period, was not associated with adverse effects in mice.

XIII.D.3 Mutagenicity and Genotoxicity

In a GLP study, an Oligonol-like product was tested in a reverse mutation test in *S. typhimurium* strains TA98, TA100, TA104, TA1535, and TA1537 with or without metabolic activation at concentrations increasing from 156 to 5,000 µg/mL. This Oligonol-like product also was tested in *E. coli* strain wp2uvra at concentrations increasing from 156 to 5,000 µg/mL. All tests were negative and the authors concluded that the Oligonol-like product is not mutagenic (Fujii *et al.,* 2007).

XIII.D.4 Human Studies

In a study conducted by Fujii *et al.* (2007), volunteers (15 men and 15 women) aged 23 to 62 years were randomly divided into 3 groups (5/sex/group) and given capsules containing either 200 mg grape seed extract (as control), 100 mg of an Oligonol-like product, or 200 mg of an Oligonol-like product to be taken daily for a period of 92 days (Fujii *et al*., 2007). Blood and urine samples were collected on Days 0, 28, and 92 for the measurement of haematology parameters (white blood cells, red blood cells, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, and platelets); liver function [glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), ALP, and GGPT]; diabetic index; fasting-blood glucose; lipid index (total cholesterol, high-density lipoprotein cholesterol, and triglycerides);

and renal function (uric acid, BUN, and creatine). All the measured parameters were within normal ranges and did not indicate any signs of toxicity.

XIII.E Safety of the Green Tea Extract Component of Oligonol®

Product-specific studies conducted with Oligonol® and the Oligonol®-like product clearly indicate a lack of adverse findings, with no toxicity observed at levels up to 1,000 mg/kg body weight/day in 90-day oral toxicity studies in rodents, no evidence of genotoxicity/ mutagenicity, and no adverse effects reported in humans at doses up to 600 mg/day. However, due to the fact that green tea catechins, especially EGCG, have been scrutinised for potential liver toxicity, and that these catechins are a minor constituent of Oligonol® (accounting for approximately 16% of the product), Amino Up undertook a thorough evaluation of the literature pertaining to the adverse effects of green tea extracts to determine their implication, if any, on the safety of Oligonol®.

XIII.E.1 14-Week Toxicity Study of Green Tea Extract

Recently, preparations of green tea extracts and/or its individual components have been marketed in a number of dietary supplements and promoted for its many reported health benefits. However, case reports of hepatotoxicity from use of these concentrated, purified forms of green tea have been identified primarily in females for weight management purposes, which have raised concerns over their safety despite the long history of safe consumption of green tea (Bonkovsky *et al.*, 2006; Sarma *et al.*, 2008; Mazzanti *et al.*, 2009). Evidence from animal studies suggests that green tea catechins, particularly EGCG, may be associated with hepatotoxicity, especially when it is administered during the fasting state (Isbrucker *et al.*, 2006; Kapetanovic *et al.*, 2009). Given that green tea extracts have been marketed for use as chemopreventative agents, the recent published adverse liver effects led the National Cancer Institute to recommend EGCG for toxicity testing. Since purified forms of EGCG are costly, and that human exposure through supplemental form occurs mostly through consumption of green tea extracts, the National Toxicology Program (NTP) decided to conduct toxicological evaluations using a green tea extract preparation (containing 48.4% EGCG, 12.8% ECG, 4.6% gallocatechin gallate, 2.26% EGC, 2.83% EC, 0.51% catechin, 0.45% catechin gallate, and 5% caffeine), rather than a purified EGCG material.

The NTP therefore conducted a 14-week toxicity study where a green tea extract was administered *via* oral gavage at doses of 0 (control), 62.5, 125, 250, 500, and 1,000 mg/kg body weight/day to F344 rats (10/sex/group) and B6C3F1 mice (10/sex/group) for 14 weeks (Chan *et al.*, 2010). In mice, the highest dose of green tea extract produced mortality in 6 males and 4 females before the end of the study, with the cause of death determined to be liver necrosis. Evidence of hepatoxicity was also observed in some of the female rats administered the highest dose of the green tea extract. On this basis, the authors determined the NOAEL for liver toxicity to be 500 mg green tea extract/kg body weight/day in both rats and mice. In addition, both rats and mice developed lesions in the nasal cavity over the course of the study. The authors determined a NOAEL for nasal toxicity at

62.5 mg/kg body weight/day for male rats, while a NOAEL for nasal toxicity could not be determined for female rats or mice of both sexes (Chan *et al.,* 2010). Evidence of nasal toxicity has not been previously reported following the oral administration of green tea extracts. Although nasal toxicity is not commonly observed for compounds administered *via* non-inhalation routes, the authors acknowledged that, in contrast to humans, the rodent nasal mucosa is an organ enriched with cytochrome P450 enzymes, as well as other xenobiotic metabolising enzymes that can potentially contribute to the metabolism of systemically absorbed compounds within this tissue (Chan *et al.*, 2010), which is likely to have impacted the effects noted following green tea extract administration.

XIII.E.2 Nasal Toxicity Study with Oligonol®

Gavage Study #3

In light of the nasal toxicity observed in a 90-day study conducted by the NTP in which green tea extract was administered to rats and mice by gavage, an additional study was conducted to evaluate the effect of Oligonol® administration on the nasal cavity since this tissue was not collected in the previous 90-day studies described above in Section IV.C.2. Sprague-Dawley rats (10/sex/group) at 5 weeks of age were administered 0 (control), 100, 300, and 1,000 mg/kg body weight/day Oligonol® by gavage once daily for a period of 13 weeks (Kitadate *et al.*, 2013). The study was conducted in accordance with GLP. Rats were acclimatised for 7 days prior to initiation of treatment and were provided with a commercial pelleted diet and tap water *ad libitum*. The animals were observed once daily for clinical signs and twice daily for mortality and moribundity. Body weights were recorded prior to treatment, once weekly during treatment, 1 day prior to necropsy and on the day of necropsy, while daily food intake was calculated from the amount consumed every 7 days. Necropsy was conducted and gross abnormalities were recorded. The nasal cavity was collected from all of the animals for histopathological examination.

There were no mortalities observed, and the only treatment-related clinical sign was compound-coloured stool in 2 of the males in the mid-dose group, and in all of the animals of the high-dose group. Treatment with Oligonol® did not affect body weight throughout the study, though males in the high-dose group had significantly higher food intake (by 7 to 13%) during Weeks 5, 6, and 11 of the study compared to controls. However, given the increased food consumption occurred transiently, and there was no significant differences in food intake in the other 90-day rat study administered 1,000 mg/kg body weight/day of Oligonol®, these changes were not considered to be toxicologically meaningful. No gross abnormalities were reported in any of the animals upon examination at necropsy. Minor microscopic changes were reported in the nasal cavity, though these occurred at similar frequency and severity in both the controls and treated animals. Thus, they were considered to be incidental findings and not toxicologically relevant. Based on these results, the authors concluded that treatment with Oligonol® does not produce toxicity in the nasal cavity at doses up to 1,000 mg/kg body weight/day.

XIII.F Summary of Studies Supporting the Safety of Oligonol®

The safety of Oligonol® has been established through the conduct of acute and sub-chronic toxicity studies in rats and mice, as well as the standard battery of genotoxicity assays (including an *in vivo* micronucleus assay, a bacterial reverse mutation test, and *in vitro* chromosome aberration assay), and 3 human studies including over 100 subjects. The oral LD_{50} in the rat was determined to be greater than 2,000 mg/kg body weight, indicating that Oligonol® is not acutely toxic. The NOAEL from 2 conventional 90-day oral toxicity studies conducted in rats is at least 1,000 mg/kg body weight/day, as no toxicologically relevant adverse effects were observed even at the highest dose tested. Likewise, there were no signs of genotoxicity/mutagenicity when Oligonol® was tested using the standard battery of genotoxicity/mutagenicity assays. No adverse events were observed when Oligonol® was administered to healthy human subjects at dosages of 600 mg/day for 14 days, or 400 mg/day for 3 months, and at 200 mg/day for 60 days in subjects with pre-hypertension or hypertension. Furthermore a 90-day study designed to specifically address the potential effect on the nasal cavity indicated that unlike the green tea extract used within the NTP 14-week study, no nasal toxicity was evident at dosages up to 1,000 mg/kg body weight/day. The lack of any toxicological safety concerns with Oligonol® were corroborated in a series of studies conducted with an Oligonol®-like product with a similar composition profile.

Together, these results suggest that Oligonol® is not expected to pose safety concerns under its intended conditions of use.

OVERALL CONCLUSION

Amino Up wishes to market Oligonol®, a 5:1 mixture of extracts from the lychee fruit (*Litchi chinensis* Sonn.) and green tea leaves [*Camellia sinensis* (L.) Kuntze], as an ingredient in conventional foods and food supplements. Oligonol® is manufactured using an oligomerisation process whereby the polyphenols present in the lychee fruit and green tea leaf extracts are cleaved into lower molecular weight oligomers and monomers. As such, Oligonol® consists mainly of monomeric flavan-3-ols, [*i.e.*, (+)-catechin, (-)-epicatechin, (-)-epicatechin 3-gallate, and (-)-epigallocatechin gallate], which combined constitute approximately 16% of Oligonol®, as well as oligomeric procyanidins formed from the condensation of these monomeric units, which constitutes approximately 76 to 80% of Oligonol®. Oligonol® is manufactured in accordance with GMP for dietary supplements, a standard that is based on guidelines prepared by the Japan Ministry of Health, Labour and Welfare and is certified by the Japan Health and Nutrition Food Association. Oligonol® is also manufactured in accordance with ISO 9001:2008 and ISO 22000:2005. Batch analysis demonstrates that the manufacturing process produces a consistent product that meets the defined specifications, and that there are no potential contaminants of concern present in the final product.

Using the EFSA FAIM tool, the highest intake level of Oligonol® on a per kg body weight basis under the proposed conditions of use in conventional foods was observed in toddlers, with mean intakes estimated at up to 14.3 mg/kg body weight/day and “heavy level” intakes estimated at up to 27.1 mg/kg body weight/day. Using reference body weights for the European population (EFSA, 2012a), the highest intake level of Oligonol® on an absolute basis was observed in adults, with mean intakes estimated at up to 616 mg/day and “heavy level” intakes estimated at up to 1,085 mg/day. The proposed use of Oligonol® in food supplements is up to 200 mg/day. Under the worst-case scenario where Oligonol® food supplements are consumed at the maximum recommended levels by adults with the highest estimated “heavy level” intake of Oligonol® from the proposed food uses, the total exposure to Oligonol® is estimated at 1,285 mg/day (18.4 mg/kg body weight/day). However, these intake estimates are likely to be grossly overestimated since it is very unlikely that individuals with the highest estimated “heavy level” intake of Oligonol® from food uses would seek to increase their dietary intake by also consuming Oligonol® food supplements,

Both lychee fruit and green tea have a long history of safe consumption in the diet. Furthermore, the polyphenol constituents in Oligonol® (*i.e.*, monomeric flavan-3-ols and procyanidins) are widely consumed in the diet through plant-derived food sources. Additionally, the safety of Oligonol® is supported by product-specific toxicology data generated from an acute oral toxicity study, 90-day oral toxicology studies in rats and mice, and a battery of mutagenicity/genotoxicity assays. Oligonol® has a low order of acute oral toxicity, with the LD_{50} reported at >2,000 mg/kg body weight. Based on the results of two 90-day oral toxicity studies in rats, the NOAEL was determined to be 1,000 mg/kg body weight/day, the highest dose tested, in both males and females. No evidence of mutagenicity/genotoxicity was observed for Oligonol®, and its safety can be further

supported by the results of several human studies in which no adverse effects were observed in subjects consuming 200 to 600 mg/day of Oligonol® supplements for as long as 3 months. Data from oral toxicity studies (*i.e.*, acute and short-term) and human clinical studies conducted with an Oligonol®-like product, which is manufactured using the same oligomerisation process but from different starting materials and is similar in composition as Oligonol®, can be used to corroborate the safety of Oligonol®.

The safety margin for the proposed use levels of Oligonol® can be calculated using based on the NOAEL of 1,000 mg/kg body weight/day, the highest dose tested in product-specific 90-day oral toxicity studies in rats. In adults, the safety margin is at least 114-fold for the estimated mean intakes of Oligonol® (1.7 to 8.8 mg/kg body weight/day), and at least 65-fold based on the estimated "heavy level" intakes of Oligonol® (3.1 to 15.5 mg/kg body weight/day), based on the proposed food uses. In toddlers, who had the highest estimated intake levels of Oligonol® on a per kg body weight basis, there is at least an 70-fold safety margin based on the estimated mean intakes of Oligonol® (3.3 to 14.3 mg/kg body weight/day), and at least 37-fold safety margin based on the estimated "heavy level" intakes of Oligonol® (11.1 to 27.1 mg/kg body weight/day). It should be noted that Oligonol® will not be added to foods that are intended for toddlers, and exposure in this population group will be limited. Oligonol® is also intended for use in food supplements at levels of 200 mg/day; there is a 54-fold safety margin for the total exposure to Oligonol® estimated under the worst-case scenario where Oligonol® food supplements are consumed at the maximum recommended levels by adults with the highest estimated "heavy level" intake of Oligonol® from the proposed food uses (*i.e.*, 18.4 mg/kg body weight/day). Although some of the safety margins are less than the generally accepted level of 100-fold, it is important to note that these safety margins are the worst-case scenario estimates. There is a large range in the exposure estimates calculated using the EFSA FAIM Tool (*i.e.*, approximately 3- to 5-fold difference), and the safety margins were calculated using the highest intake levels of Oligonol® observed across a range reported based on data from various dietary surveys collected in 17 different European countries. Furthermore, all foods containing Oligonol® are assumed to be consumed at the maximum proposed use levels; therefore, the actual intake of Oligonol® from the proposed conditions of use will be less than anticipated. Moreover, it is very unlikely that individuals with the highest estimated "heavy level" intake of Oligonol® from food uses would seek to increase their dietary intakes by also consuming food supplements containing Oligonol®. In light of these considerations, and the fact that the NOAEL of 1,000 mg/kg body weight/day represents the highest dose tested in product-specific 90-day toxicity studies, the proposed food and food supplement uses of Oligonol® can be justified.

While product-specific studies conducted with Oligonol® clearly supports its use as a food ingredient in the stipulated food categories, due to the green tea extract component of the product, Amino Up conducted a close evaluation of the literature pertaining to the published adverse effects of green tea catechins so as to confirm the safety of the Oligonol® product. Cases of hepatotoxicity have been reported following consumption of dietary supplements containing highly concentrated, purified green tea extracts. These liver effects have subsequently been corroborated, along with the novel finding of nasal toxicity, in a 14-week

oral toxicity study conducted by the NTP where a specific green tea extract preparation was administered by gavage to rodents. However, these findings are not considered relevant to the safety of Oligonol®. A close examination of the serum biochemistry and pathology data from the two standard 90-day toxicity studies conducted in rats revealed no adverse findings that would be suggestive of liver toxicity following administration of Oligonol® at doses up to 1,000 mg/kg body weight/day. Similarly, clinical chemistry data from the 3 human studies did not indicate any changes in liver function in subjects consuming Oligonol® at doses up to 600 mg/day. To address the potential concerns regarding nasal toxicity, it was established in another rat subchronic study that there were no histopathological changes to the nasal cavity following administration of Oligonol® by gavage at dosages up to 1,000 mg/body weight/day for 90 days, thereby confirming that the effects noted in the 14-week toxicity studies conducted by the NTP were not relevant to Oligonol®. Furthermore, the amount of green tea catechins that would be consumed from the proposed uses of Oligonol® is comparable to the amount obtained from one 200 mL serving of green tea. Therefore, the green tea extract component of Oligonol®, which represents only approximately 17% of the final product, is not expected to pose any safety concerns.

Collectively, the scientific evidence presented herein demonstrates that Amino Up's Oligonol® ingredient would not produce adverse effects on human health under the proposed conditions of use in conventional foods and food supplements.

GLOSSARY

ADME	Absorption, Distribution, Metabolism, and Elimination
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ANS	Additive and Nutritive Sources
AST	aspartate aminotransferase
BUN	blood urea nitrogen
CAS	Chemical Abstract Service
GMP	Good Manufacturing Practice
ECG	(-)-epicatechin 3-gallate
EFSA	European Food Safety Authority
EGC	(-)-epigallocatechin
EGCG	(-)-epigallocatechin gallate
EU	European Union
FAIM	Food Additives Intake Model
FCS	Food Classification System
GGPT	*gamma*-glutamyl transpeptidase
GLP	Good Laboratory Practice
GM	genetically modified
GOT	glutamic-oxaloacetic transaminase
GPT	glutamic pyruvic transaminase
GRAS	Generally Recognized as Safe
HPLC	high-performance liquid chromatography
LD_{50}	median lethal dose
NDI	New Dietary Ingredient
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
OECD	Organisation for Economic Co-operation and Development
ROS	reactive oxygen species
SCF	Scientific Committee on Food
U.S.	United States
UK	United Kingdom

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