Application for the Approval of Bonolive® (standardised olive leaf extract)

Under

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

Non-Confidential Dossier

15 December 2016

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ADMINISTRATIVE DATA

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EXECUTIVE SUMMARY

Approval is sought under Regulation (EC) No 258/97 of the European Parliament and of the Council of 27th January 1997 concerning novel foods and novel food ingredients, for a standardised olive leaf extract (hereinafter referred to as Bonolive®) as an ingredient for use in functional foods, food supplements and foods for special medical purposes.

Bonolive® is a standardised extract prepared from the leaves of the olive tree (*Olea europaea* L.). The source plant is not genetically modified. As such, this ingredient falls under category (e) of Article 1(2) or Regulation (EC) No 258/97: "foods and food ingredients consisting of or isolated from plants and food ingredients isolated from animals, except for foods and food ingredients obtained by traditional propagating and breeding practices and which have a history of safe food use".

Bonolive® is standardised to contain between 40 and 55% polyphenols, with the majority of these polyphenols being oleuropein. Appropriate product specifications for identity and potential contaminants have been established for this ingredient. The results of batch analyses indicate that the manufacturing process produces a consistent product meeting the product specifications. Analysis also demonstrates that heavy metal, microbial, and pesticide levels are below detection thresholds. Furthermore, the results of stability studies indicate that Bonolive® is stable for at least 60 months.

Bonolive® is manufactured in accordance with the food grade and safety standards of the Global Food Safety Initiative under the Food Safety System Certification 22000, which includes all elements of Good Manufacturing Practices and Hazard Analysis Critical Control Points, and thus covers European Union (EU) hygiene requirements [*i.e*., Regulation (EC) 852/2004]. Bonolive® is produced by aqueous extraction, followed by concentration, purification and vacuum drying. These methods are typical of the food industry and are not anticipated to result in any toxicological, nutritional, or microbiological hazards. Neither the raw materials nor processing aids used in the production of Bonolive® have been genetically modified.

The olive tree is cultivated in a number of areas throughout the world. The polyphenol compounds from the olive tree have been consumed for millennia and while the olive fruit is the most commonly consumed part of the olive tree, the leaves of olive trees, and extracts thereof, have also been traditionally consumed in the EU and olive leaf extracts are widely used in food supplements in the EU.

Bonolive® is proposed for use as an ingredient in a range of food categories, including yoghurts, fine bakery wares and beverages. To that purpose, Bonolive® has been developed as a highly water-soluble extract with a high polyphenol concentration that can be easily used at low dosages in all the afore-mentioned food matrices. In addition, it is intended for use in functional foods, food supplements and foods for special medical purposes (FSMPs). As FSMPs are considered case by case for use in finished products, the safety and suitability within the context of the whole food would be justified in a

notification to a member state wherein the appropriate marketing requirements would be determined.

As described throughout the dossier, products containing the ingredient will clearly be targeted towards individuals (men and women) aged over 50 years that want to support their bone and joint health. Estimates for the anticipated intake of Bonolive® by the EU population have been determined using the proposed uses in combination with (1) the European Food Safety Authority Comprehensive food consumption database (EFSA Comprehensive database) and (2) the United Kingdom (UK) National Diet and Nutrition Survey (NDNS) Rolling programme Years 1-4 (2008-2012). Intakes described among adults, elderly, and very elderly population groups in the EFSA Comprehensive database [wherein the mean intakes ranged from 0.1 to 6.9 mg/kg body weight/day (equivalent to 7 to 483 mg/day) and the high level intakes ranged from 0.5 to 30.1 mg/kg body weight/day (equivalent to 38 to 2,107 mg/day)], are considered the most pertinent estimates of anticipated dietary exposure within the intended target population when calculated using this screening tool. Considering the average "maximum" use level of Bonolive®, of 179 mg/ serving, these intakes are equivalent to between <1 to 3 servings/day at the mean and between <1 to 12 servings/day at the high level. The upper range of these high level intakes were considered to be unrealistic (*i.e.*, that an individual would chronically consume 12 servings per day of products containing Bonolive®), as such, it was deemed necessary relevant to conduct a more refined assessment. The UK NDNS dataset permits analysis by the 'target population' alone, *i.e*., individuals 50 years and over that want to support their bone and joint health. Using this dataset, mean and 95th percentile intakes were calculated at 0.5 and 1.7 mg/kg body weight/day (equivalent to 41 and 131 mg/day, respectively). Considering the average "maximum" use level for the two main contributing categories (fruit juices and yoghurts) of 32.5 mg/serving, the intake values in this age group are equivalent to just over 1 serving/day at the mean, and 4 servings/day at the 95th percentile; these are expected to be the most accurate estimates of chronic dietary exposure to this ingredient by consumers. These NDNS values were used to determine cumulative exposure from foods and beverages, as well as food supplements containing Bonolive®; thereby assuming that an individual is a high consumer of all foods containing this ingredient, as well as a regular consumer of food supplements. The resulting mean and 95th percentile intakes were calculated at 291 and 381 mg/person/day, respectively; this is equivalent to 4.1 and 5.3 mg/kg body weight/day for a 70 kg adult. Finally, Bonolive® is proposed for use in foods for special medical purposes at a maximum dose of 625 mg/day. In accordance with the requirements of Directive 1999/21/EC (in force until February 2019), these products will be notified before being placed on the market. These products would be targeted only towards adults of 50 years and older, and the daily intake of Bonolive® would be limited to only these products in the diet; as such, for a 70 kg adult, the corresponding intakes would be 8.9 mg/kg body weight/day.

Bonolive® is not nutritionally equivalent to other foods and is not intended to replace other foods currently on the market, apart from to supplement the natural polyphenols from the "Mediterranean diet". The product is intended for a population of 50 years and will be used

in health foods and supplements targeting that segment of the population. Such premium products are typically sold in specialised retail channels, such as pharmacies and health stores and will typically carry labelling with specific recommendations for use (*e.g.* recommended daily dosage). Given the positioning for the target group, the premium pricing, the specific serving formats and labelling and the specialised retail channels, it is highly unlikely that such products will be consumed by people outside target group.

Bonolive® is not anticipated to adversely impact the quality of the diet. Bonolive® is comprised of 94.8% carbohydrates, of which a significant part is polyphenols, with only minimal amounts of fat (0.2%) and protein (1.16%).

The safety of Bonolive® is supported by product specific preclinical and clinical studies, as well as non-product-specific studies on various olive leaf extracts and powders. Metabolic fate data demonstrate that the phenolic compounds are highly bioavailable and the absorption, metabolism, and renal clearance of phenolic compounds from olive leaves is relatively rapid. Following a 14-day dose range-finding oral toxicity study, a 90-day oral toxicity study was conducted in rats using Bonolive® where the no-observed-adverse-effect level (NOAEL) for Bonolive® was determined to be 1,000 mg/kg body weight/day, the highest dose tested. The results of a bacterial reverse mutation test, an *in vitro* chromosomal aberration test, and an *in vivo* mammalian erythrocyte micronucleus test demonstrate that Bonolive® is not genotoxic. Additional non-product specific pre-clinical studies using other olive leaf extracts corroborate safety. Thus, given that the 95th percentile intakes of Bonolive® from its cumulative exposure from foods and beverages, as well as food supplements containing Bonolive®, for the target population in adults (based on the refined assessment using the UK NDNS) is 5.3 mg/kg body weight/day, there exists a 188-fold safety factor compared to the NOAEL of 1,000 mg/kg body weight/day, the highest dose tested. In the non-target population, a worst-case 95th percentile intakes of 9.3 mg/kg body weight/day (in children) was estimated, assuming that 100% of foods and beverages in which Bonolive® is proposed for use contained the ingredient at the maximum use level and that all these foods were consumed by the non-target population. As mentioned above, these products would, in the worst case, only be consumed incidentally by children and younger age groups. Even in this worst-case hypothetical scenario, the margin of safety remains sufficient, at 108. These intake levels are further supported by clinical studies using Bonolive® which demonstrate that this ingredient is well tolerated and without serious adverse effects in humans at doses of at least 250 mg/day. Additional non-product specific clinical studies using other olive leaf extracts corroborate the tolerability of Bonolive®.

Furthermore the worst case exposure of olive polyphenols from Bonolive® is well within that consumed from olives and olive oil per day as part of the “Mediterranean diet”.

Collectively, the scientific evidence presented herein demonstrates that BioActor’s Bonolive® would not produce adverse health effects on human health under the intended conditions of use in functional foods and beverages, and food supplements.

GENERAL INTRODUCTION

Bioactor B.V. (Bioactor) proposes to market a high olive polyphenol olive leaf extract, derived from the leaves of *Olea europaea* L. and standardised to contain a minimum of 40% polyphenols, the primary polyphenol being oleuropein (hereinafter referred to as Bonolive®), for use as a food ingredient in several food categories in Europe. Approval is sought under Regulation (EC) No 258/97[1] of the European Parliament and of the Council of 27th January 1997 concerning novel foods and novel food ingredients (hereafter referred to as EC 258/97), and accordingly, this submission has been prepared pursuant to the Commission Recommendation of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients (hereafter referred to as the Commission Recommendation of 1997).

Article 1(2.) of EC 258/97 states that the regulation "…shall apply to the placing on the market within the Community of foods and food ingredients which have not hitherto been used for human consumption to a significant degree within the Community and which fall under the following categories…(e) foods and food ingredients consisting of or isolated from plants and food ingredients isolated from animals, except for foods and food ingredients obtained by traditional propagating and breeding practices and which have a history of safe food use". Bonolive® is thus considered a novel food/food ingredient due to the isolation and concentration of the product from its source.

Section 4 of the Commission Recommendation of 1997 outlines recommendations made by the Scientific Committee on Food (SCF) concerning to the "Scientific Classification of Novel Foods for the Assessment of Wholesomeness", which facilitates the safety and nutritional evaluation of a given novel food/food ingredient. Of the 6 classes identified, Bonolive® would be classified in Class 2 as a "complex NF from non-GM source", since the production of Bonolive® is conducted using conventional techniques, and with no use of genetic modification. The olive plant has a history of use in the community. Accordingly, Bonolive® would be further allocated under Sub-Class 2.1: "the source of the novel food has a history of food use in the Community". The essential information requirements corresponding with this classification are outlined in a detailed list below, and are expanded upon in separate sections throughout the document, forming the basis of the application (Recommendation 97/618/EC[2]).

[1] Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. OJ L 43, 14.2.1997, p. 1–6.
[2] 97/618/EC: Commission Recommendation of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients and the preparation of initial assessment reports under Regulation (EC) No 258/97 of the European Parliament and of the Council. OJ L 253, 16.9.1997, p. 1–36.

I Specification of the Novel Food

II Effect of the Production Process Applied to the Novel Food

III History of the Organism Used as the Source of the Novel Food

IV-VIII Not Applicable

IX Anticipated Intake/Extent of Use of the Novel Food

X Information from Previous Human Exposure to the Novel Food or its Source

XI Nutritional Information on the Novel Food

XII Microbiological Information on the Novel Food

XIII Toxicological Information on the Novel Food

For each category (I through XIII), structured schemes have been developed by the SCF, which consist of a decision-tree-like set of questions designed to elicit sufficient data for a comprehensive safety and nutritional evaluation of the novel food. As outlined below in Sections I through XIII, the required questions are identified and subsequently addressed with the appropriate data. Following the release of the new novel foods Regulation (EU) 2015/2283[3] (which comes into force January 1, 2018), EFSA has developed *Guidance on the preparation and presentation of an application for authorisation of a Novel Food in the Context of Regulation (EU) 2015/2283* as adopted on September 21, 2016 and published on November 10, 2016 which prescribes a common format for the organisation of the information to be presented in an EU novel food submission (EFSA, 2016). Since this novel food application may fall under the new regulations, should the regulatory review process continue past January 1, 2018, a cross reference table that outlines which sections of this dossier cover the data requirements as listed in the EFSA guidance is presented below in Table 1. Bonolive® will fall under Article 2 Definition 2 category (iv) “food consisting of, isolated from or produced from plants or their parts,…” of Regulation (EU) 2015/2283 and as such, the applicable sections for this definition were referred to in the guidance document.

[3] Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001. OJ L 327, 11.12.2015, p. 1–22.

Table 1 Cross Reference Table Bridging European Union Novel Food Dossier Requirements

Commission Recommendation of 1997[1]	EFSA Guidance According to Regulation (EU) 2015/2283 [2]	Comments
I Specification of the Novel Food	1. Description of the Novel Food 3. Compositional data 4. Specifications	Taxonomic classification summarised in III.C
II Effect of the Production Process Applied to the Novel Food	2. Production process	N/A
III History of the Organism Used as the Source of the Novel Food	5. History of use of the Novel Food and of its source 5.1 History of the source	N/A
IV-VIII Not Applicable	N/A	N/A
IX Anticipated Intake/Extent of Use of the Novel Food	6. Proposed uses and use levels and anticipated intake	N/A
X Information from Previous Human Exposure to the Novel Food or its Source	5. History of use of the Novel Food and of its source 5.1 History of the source 5.2 History of use of the Novel Food	N/A
XI Nutritional Information on the Novel Food	8. Nutritional information	N/A
XII Microbiological Information on the Novel Food	(covered under part 1)	N/A
XIII Toxicological Information on the Novel Food	7. Absorption, distribution, metabolism, and excretion (ADME) 9. Toxicological information 10. Allergenicity	N/A

EFSA = European Food Safety Authority; N/A = not applicable

[1] http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:31997H0618&from=EN

[2] http://www.efsa.europa.eu/en/efsajournal/pub/4594

I SPECIFICATIONS OF Bonolive®

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

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

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

I.A Identity

I.A.1 Common Name or Usual Name

Olive Leaf Extract, standardised to contain a minimum of 40% polyphenols (predominantly oleuropein)

Trade name: Bonolive®

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

Not available for Bonolive®. The characteristic compound, oleuropein, is identified by the CAS number 32619-42-4.

I.A.3 Description

Bonolive® is a standardised extract prepared from the cut leaves of *Olea europaea* L. (the common olive tree).

I.A.4 Composition

Nutritional analysis of Bonolive® demonstrates an energy content of 386 kcal/100 g (1638 kJ/100 g). Bonolive® only contains minimal amounts of fat (0.2%), protein (1.16%), and ash (1.67%) with the major fraction identified as carbohydrates (94.8%). A large fraction of the carbohydrates are polyphenols and the extract is standardised to contain a minimum of 40% oleuropein (the primary polyphenol), up to a maximum of 55%. The nutritional composition of Bonolive® is presented below in Table I.A.4-1 and the composition of the polyphenol component (with oleuropein representing 40 to 55% of the extract) is presented in Table I.A.4-2, demonstrating that oleuropein (a secoiridoid) comprises the large majority of the polyphenols in Bonolive® (approximately 80% based on a chromatographic profile). The data included in Table I.A.4-2 are based on analysis of 1 batch of Bonolive®; analysis reports are available in Appendix A.

Table I.A.4-1 Nutritional Composition of Bonolive®

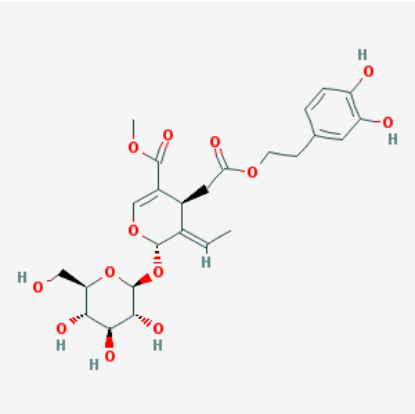
Component	Percentage
Total Fat	0.2%
Carbohydrates	94.8%
Oleuropein	≥40%
Fibre	<0.1%
Ash	1.67%
Moisture	2.2%
Protein	1.16%
Sodium	0.172%
Sugars	<1%

Table I.A.4-2 Typical Polyphenol Composition of Bonolive®	
Component	**Percentage of Total Polyphenols**
Oleuropein	83.88%
Hydroxytyrosol glucoside	0.17%
Oleoside	3.25%
Hydroxytyrosol	1.72%
Oleoside-11-methyl ester	3.59%
Demethyloleuropein	0.97%
Verbascoside	2.14%
Rutin	0.60%
Apigenin-7-glucoside	0.40%
p-HPEA-EA (tyrosol derivative)	0.32%
Luteolin-7-glucoside	2.23%
3,4-DHPEA-EA (hydroxytyrosol derivative)	0.54%
Luteolin	0.19%

I.A.5 Molecular Structure

This is not applicable for a complex extract such as Bonolive®. The molecular structure of oleuropein, the primary polyphenol in Bonolive®, is presented below in Figure I.A.5-1.

Figure I.A.5-1 Molecular Structure of Oleuropein



I.B Product Specifications

The product specifications for Bonolive® are presented below in Table I.B-1. Methods of analysis for identification, total and individual heavy metals, and microbiological endpoints are according to the European Pharmacopoeia.

Table I.B-1 Specifications for Bonolive®		
Specification Parameter	**Specification**	**Reference/Test Methodology Performance of Test**
Identification		
Appearance	Yellow to brown powder	Internal
Solubility	Freely soluble in water	Ph.Eur. definition
Loss on drying	≤8%	Ph.Eur.2.2.32.
Residue by calcination	≤9%	Ph.Eur.2.4.14.
Olive polyphenols (=oleuropein)	40–55%	Internal method based on Ph.Eur.2.2.29 (see Appendix B)
Heavy Metals		
Total heavy Metals	≤20 mg/kg	Ph. Eur. 2.4.8 (and UNE-EN 13805)
Lead	≤0.5 mg/kg[1]	Ph.Eur. method 2.4.27 & Ph.Eur.2.2.58 (and UNE-EN 13805 & 15763)
Cadmium	≤1 mg/kg[1]	Ph.Eur. method 2.4.27 & Ph.Eur.2.2.5 (and UNE-EN 13805 & 15763)
Mercury	≤0.1 mg/kg[1]	Ph.Eur. method 2.4.27 & Ph.Eur.2.2.58 (and UNE-EN 13805 & 15763)
Arsenic	≤0.3 mg/kg	Ph.Eur. method 2.4.27 & Ph.Eur.2.2.58 (and UNE-EN 13805 & 15763)
Microbiological		
Total plate count	$\leq 10^3$ CFU/g	Ph. Eur. 1.6.12 & Ph. Eur. 1.6.13
Yeast & mould	$\leq 10^2$ CFU/g	Ph. Eur. 1.6.12 & Ph. Eur. 1.6.13
Enterobacteria	$\leq 10^2$ PNB/g	Ph. Eur. 1.6.12 & Ph. Eur. 1.6.13
Salmonella	Absent/10 g	Ph. Eur. 1.6.12 & Ph. Eur. 1.6.13
Escherichia coli	Absent/1 g	Ph. Eur. 1.6.12 & Ph. Eur. 1.6.13
Staphylococcus aureus	Absent/1 g	Ph. Eur. 1.6.12 & Ph. Eur. 1.6.13

CFU = colony forming units; PNB = probable number of bacteria; UNE -EN = Una Norma Española
[1] Meets, or is more conservative than, maximum level for food supplements as specified in Commission Regulation (EC) No 1881/2006 on contaminants in foodstuffs

I.C Batch Analyses

Batch analyses for 5 batches of Bonolive® are presented below in Table I.C-1 and demonstrate that the manufacturing process produces a consistent product according to the established product specifications detailed in Section I.B. See Appendix C for certificates of analysis.

Table I.C-1 Batch Results for Bonolive®

Parameter	Specification	Batch No.				
		016F019	ID110-L38-1	ID110-L38-2	ID110-L38-3	ID110-L38-4
Identification						
Appearance	Yellow to brown powder	Complies	Complies	Complies	Complies	Complies
Solubility	Freely soluble in water	Complies	Complies	Complies	Complies	Complies
Loss on drying	≤8%	1.5%	1.2%	1.5%	1.4%	1.0%
Residue by calcination	≤9%	3.3%	1.2%	1.3%	1.3%	1.3%
Olive polyphenols (=oleuropein)	40–55%	42.0%	52.2%	53.3%	52.6%	53%
Heavy Metals						
Total heavy Metals	≤20 mg/kg	Complies	Complies	Complies	Complies	Complies
Lead[1]	≤0.5 mg/kg	Complies	Complies	Complies	Complies	Complies
Cadmium[1]	≤1 mg/kg	Complies	Complies	Complies	Complies	Complies
Mercury[1]	≤0.1 mg/kg	Complies	Complies	Complies	Complies	Complies
Arsenic[1]	≤0.3 mg/kg	Complies	Complies	Complies	Complies	Complies
Microbiological						
Total plate count	$\leq 10^3$ CFU/g	Complies	Complies	Complies	Complies	Complies
Yeast & mould	$\leq 10^2$ CFU/g	Complies	Complies	Complies	Complies	Complies
Enterobacteria	$\leq 10^2$ PNB/g	Complies	Complies	Complies	Complies	Complies
Salmonella	Absent/10 g	Complies	Complies	Complies	Complies	Complies
Escherichia coli	Absent/1 g	Complies	Complies	Complies	Complies	Complies
Staphylococcus aureus	Absent/1 g	Complies	Complies	Complies	Complies	Complies

CFU = colony forming units; PNB = probable number of bacteria
[1] Analysis conducted on the starting material

I.D Contaminants

Raw Material Related Contaminants

Specifications for heavy metal and microbial contamination are established to ensure the absence of these substances in Bonolive®. Additionally, 1 out of every 10 batches of Bonolive® are analysed for 250 different pesticide residues including those used for olive tree treatment. It should be noted that, if needed, olive trees are generally treated with pesticides in May after blossoming to preserve the olives. Bonolive® is typically manufactured using the leaves from the February pruning; thus, these leaves have during the last growing season not been exposed to treatment with pesticides, making it highly unlikely that pesticide testing would lead to positive results. The results of residual pesticide analyses of Batch No. 02C037 (provided in Appendix D) demonstrate that all pesticide residues were below detection limit.

Process Related Contaminants

Bonolive® is produced using an aqueous extraction, the water level is adjusted throughout the process.

I.E Stability

Real-time stability testing was conducted on 1 batch of Bonolive®. Standard storage conditions for Bonolive® are: "original air-tight containers to prevent dust formation, in a dry and cool place, away from direct sunlight. Keep away from ignition, heat or electricity sources." The real-time stability test was conducted on samples stored under standard storage conditions and tested annually for 5 years. The results of this test are presented below in Table I.E-1 and demonstrate that Bonolive® is stable under standard storage conditions for at least 5 years.

Table I.E-1 Real-time Stability Study for Bonolive® (Batch No. ID031-P04) Stored Under Standard Storage Conditions

Month	Appearance	Loss on drying (%)	Oleuropein (%)
0	Complies	0.9	40.9
12	Complies	1.3	42.0
24	Complies	1.2	43.5
36	Complies	1.6	40.7
48	Complies	1.4	44.4
60	Complies	1.5	42.3

II EFFECT OF THE PRODUCTION PROCESS APPLIED TO Bonolive®

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

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

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

II.A Raw Materials Used in the Manufacturing Process

II.A.1 Raw Materials and Processing Aids

The olive leaves are obtained from trees cultivated mainly in Andalusia, Spain, but also from the Ciudad Real and Albacete regions of Castilla-La-Mancha, Spain and occasionally from North Africa. The olive trees from which the leaves are taken are farmed mainly for olive/olive oil production with the trees pruned twice per year in February and August. Olive trees are generally treated with pesticides after blossoming in May. Since the leaves are obtained from the half-yearly pruning activities (for Bonolive® typically from the February pruning), those leaves have not recently been exposed to pesticides. Specifications for the olive leaves are provided in Appendix E.

A confidential processing aid also is used.

II.B Manufacturing Process

II.B.1 Description of the Manufacturing Process

Bonolive® is manufactured *via* aqueous extraction using the leaves of the olive tree (*Olea europaea* L.).

II.B.2 Control of the Manufacturing Process

Bonolive® is manufactured in accordance with the food grade and safety standards of the Global Food Safety Initiative (GFSI) under the Food Safety System Certification 22000 (FSSC 22000), which includes all elements of Good Manufacturing Practices (GMP) and Hazard Analysis Critical Control Points (HACCP), and thus covers EU hygiene requirements (*i.e.*, Regulation (EC) 852/2004[4]) together with a comprehensive management system. See FSSC 22000 certificate in Appendix F.

II.B.3 History of Use of the Manufacturing Process

Bonolive® is manufactured through the aqueous extraction of the olive leaves. Aqueous extraction is a common technique used in food processing.

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

It is not anticipated that any toxicological, nutritional, or microbiological hazards will arise from the production process.

[4] Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. OJ L 139, 30.4.2004, p. 1–54.

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

There is no anticipated potential for Bonolive® to alter the levels of substances with an adverse effect on public health.

II.E Potential Contamination of Micro-Organisms of Adverse Health Significance

Microbiological hazards are not expected to arise as a result of the production process. The higher temperatures of the evaporation and a hydrophobic purification process will aid in reducing the risk of microbiological hazards. Additionally, analytical data demonstrating the absence of microbiological contaminants are presented above in Section I.C.

III HISTORY OF THE SOURCE ORGANISM - *Olea europaea* L.

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

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

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

III.A Biological Source

Bonolive® is produced from the leaves of the *Olea europaea* L. tree (common olive). It is a dicot tree that is cultivated in several areas throughout the world and is a particularly important fruit tree in the European and African countries bordering the Mediterranean Sea, where it is commercially grown on more than 23 million acres (Vossen, 2007; Arslan and Özcan, 2011). It also is cultivated in the United States (California), as well as in Chile, Argentina, South Africa and Australia (Vossen, 2007).

III.B Derivation Using Genetic Modification

The raw materials and processing aids used in the production of Bonolive® have not been genetically modified.

III.C Characterisation of the Source Organism

Bonolive® is extracted from *O. europaea* L. The current taxonomic placement of *O. europaea* L. is summarised below[5]:

Kingdom: Plantae

Subkingdom: Tracheobionta

Superdivision: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Asteridae

Order: Scrophulariales

Family: Oleaceae

Genus: *Olea* L.

Species: *Olea europaea* L.

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

The fruit of *O. europaea L.* is widely consumed as the common olive and is used to produce olive oil (Vossen, 2007). The polyphenol compounds from the olive tree have been consumed for millennia, particularly in the Mediterranean region (Vossen, 2007). Individuals who consume the typical Mediterranean diet generally ingest up to 90 mg (± 105 mg) of polyphenols from olives per day (combined intake from olive oil [21.9 ± 10.4 mg] and olives

[5] http://plants.usda.gov/core/profile?symbol=OLEU

[68.5 ± 104 mg]) (de Bock *et al*., 2013a,b; Tresserra-Rimbau *et al*., 2013). The leaves of olive trees have also been consumed traditionally for health purposes; nineteenth century references cite olive leaf use as a febrifuge (Pallas, 1828; Hanbury, 1854). Additionally, the use of olive leaf extract in food supplements is not considered to be novel according to the European Commission Novel Food catalogue (European Commission, 2016). Moreover, per Section IX.A, one of the intended uses of Bonolive® is in food supplements. Various olive leaf extract containing products are currently sold within the EU. A non-exhaustive overview of products based on olive leaf extracts that are sold in the UK (Holland and Barrett stores), Belgium, The Netherlands, and France are presented below in Table III.D-1. As demonstrated in this table, several of these products, when taken at the recommended dosage, result in an oleuropein intake at levels that are substantially higher than the 100 mg oleuropein daily dosage recommended for Bonolive®.

Table III.D-1 Olive Leaf Products for Sale as Food Supplements in the UK, Netherlands, Belgium, and France

Company	Product Name	Serving Size
Comvita	Olive Leaf Extract	1 capsule/day, equivalent of 3.5 g fresh olive leaf, 66 mg Oleuropein/capsule
Bional	Garlic + Olive Leaf + Hawthorn	3 capsules/day 300 mg olive leaf oil macerate/ 3 capsules
Comvita	Olive leaf Complex liquid	15 mL/day Equivalent of 5 g fresh leaf per 5 mL 66 mg oleuropein/15 mL
Ovivo	Olive leaf Infusion with calendula	70 mL/day 154 mg oleuropein/70 mL
Solgar	Olive Leaf Extract	1 to 3 capsules/day 225 mg standardised olive leaf powdered extract (40 mg oleuropein)/capsules
Biovitaal	Olive Leaf Complex	1-3 capsules/day 325 mg standardised olive leaf powdered extract >20% 195 mg Oleuropein/3 capsules
Mannavita	Olive Leaf + Hibiscus	2 capsules/day 1000 mg olive leaf extract 200 mg Oleuropein
Nutrixeal	Oleurovital, Olive Leaf Extract	1-4 capsules/day 500 mg standardised olive leaf extract (30% Oleuropein) 150 mg oleuropein/capsule
Irelia	Olive leaf extract	3 capsules/day 400 mg olive leaf extract, 22% oleuropein 264 mg oleuropein/3 capsules
Fairvital	Olive leaf extract	1 capsule/day 500 mg olive leaf extract, 20% oleuropein 100 mg oleuropein/capsule

IV-VIII NOT APPLICABLE

IX INTAKE/EXTENT OF USE OF Bonolive®

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

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

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

IX.A Intended Uses of Bonolive®

Bonolive® is currently proposed for use in the EU in specific food categories, as summarised in Table IX.A-1.

Table IX.A-1 Summary of the Individual Proposed Food and Beverage Uses and Use-Levels for Bonolive® in the European Union

Group No.	EU FoodEx Level 1 Group	Specific Uses	Maximum Proposed Use Level per Serving (mg/serving)	Typical Serving (g or mL)[1]	Maximum Proposed Use Level (g/100 g)
1	Grains & grain-based products	Cereal and granola bars	125	30	0.4
		Nutrition bars			
8	Milk and dairy products	Yoghurts, flavoured only	25	125	0.02
		Flavoured milk drinks	125	200	0.0625
10	Sugar and confectionery	Gummies	332	40	0.83
		Chewing gum	167	2	8.33
18	Products for special nutrition use	FSMPs	Case by case basis, max. 625 mg/day	NA	Case by case basis, max. 625 mg/day
12	Fruit and vegetable juices	Fruit juice	40	200	0.02
		Fruit nectars and similar[2]			
		Vegetable juices			
14	Non-alcoholic beverages	Fortified water	125	250	0.05
		Energy and sports drinks (incl. powdered forms)			
		Nutrition drink			
17	Food supplements as defined in Directive 2002/46/EC, excluding supplements for infants and young children	Food supplements	250 mg/day	NA	250 mg/day

EU = European Union; FSMPs = foods for special medical purposes
[1] FSA Food Portion Sizes, Third Edition (FSA, 2002)
[2] No food codes were identified in the UK NDNS for 'fruit nectars', as such, fruit smoothies were selected as a surrogate for this food use.

As noted in Table IX.A-1, one of the proposed food-uses for Bonolive® is in foods for special medical purposes (FSMPs). As such, Bonolive®, as an ingredient in FSMPs, will be regulated under Regulation (EU) No 609/2013[6]. FSMPs containing Bonolive® will only be used under medical supervision and the labelling of these foods shall be compliant with requirements specified in Article 4 of Directive 1999/21/EC[7], and Regulation (EU) No 2016/128[8] when it enters into force on 22 February 2019. Further, prior to marketing, the food business operator will notify each Member State in which the FSMP is intended to be sold, as per the requirements of these regulations.

IX.B Anticipated Daily Intakes of Bonolive®

Estimates for the anticipated intake of Bonolive® (olive leaf extract) by the EU population have been determined using the proposed uses and use levels (see Table IX.A-1) in combination with food consumption data from the following two resources, in line with the recommendations of the most recent EFSA guidance on novel food dossier application preparation (EFSA, 2016):

1. The European Food Safety Authority Comprehensive Food Consumption Database[9] (EFSA Comprehensive database hereafter) was used a screening tool; and

2. Refined estimates were determined using individual food consumption data collected as part of the most recently available release from the United Kingdom (UK) National Diet and Nutrition Survey (NDNS) Rolling programme, 2008-2012 (Department of Health, 2014; NatCen Social Research, MRC Human Nutrition Research, University College London, Medical School, 2015)[10].

Full details on these datasets are provided in Appendix G, along with the full intakes assessment report; the main results are summarised for both datasets in the sections that follow. Bonolive® is intended for consumption by individuals over 50 years of age, however, for completeness sake (and to ensure the safety for younger age groups, who may incidentally be exposed to foods and beverages containing this ingredient), intakes assessments have been conducted for all population groups, namely:

[6] Regulation (EU) No 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control and repealing Council Directive 92/52/EEC, Commission Directives 96/8/EC, 1999/21/EC, 2006/125/EC and 2006/141/EC, Directive 2009/39/EC of the European Parliament and of the Council and Commission Regulations (EC) No 41/2009 and (EC) No 953/2009. OJ L 181, 29.6.2013, p. 35-56.
[7] Commission Directive 1999/21/EC of 25 March 1999 on dietary foods for special medical purposes. OJ, L 91, 7.4.1999, p. 29-36.
[8] Commission Delegated Regulation (EU) 2016/128 of 25 September 2015 supplementing Regulation (EU) No 609/2013 of the European Parliament and of the Council as regards the specific compositional and information requirements for food for special medical purposes. OJ L 25, 2.2.2016, p. 30-43.
[9] http://www.efsa.europa.eu/en/food-consumption/comprehensive-database
[10] https://www.gov.uk/government/statistics/national-diet-and-nutrition-survey-results-from-years-1-to-4-combined-of-the-rolling-programme-for-2008-and-2009-to-2011-and-2012 ; https://discover.ukdataservice.ac.uk/doi?sn=6533#6

- infants, ages up to 11 months (EFSA Comprehensive only);
- toddlers, ages 12 months (18 months for UK NDNS) up to 35 months;
- children, ages 3 to 9 years;
- teenagers, ages 10 to 17 years;
- adults, ages 18 to 64 years;
- pregnant women (EFSA Comprehensive only);
- lactating women (EFSA Comprehensive only);
- elderly, ages 65 to 74 years;
- very elderly, aged 75 years and older;
- target UK population, ages 50 years and older (UK NDNS only); and
- total UK population, all ages (UK NDNS only).

IX.B.1 EFSA Comprehensive Dataset

IX.B.1.1 Estimated Daily Intakes

Mean and high level intakes of Bonolive® as calculated for EU Member States were calculated using consumption data from the EFSA Comprehensive database on a per kilogram body weight basis, which are presented as a range from lowest to highest for each population group in Table IX.B.1-1 below. These estimated values were converted to an absolute basis (mg/day) using EFSA default body weights (EFSA, 2012).

It is not possible to directly compare the intake results across countries due to the different methodologies used in separate surveys; however, some overall observations can be made based on the range of estimated intakes. The highest mean intakes of Bonolive® on a body weight basis were observed in toddlers and other children, which is expected based on the relatively higher intake of foods and beverages on a body weight basis when compared to the rest of the population (EFSA, 2011a). In the remaining age classes (infants; and from adolescents to the very elderly), mean intakes remained lower than 7.5 mg/kg body weight/day among all the countries examined. High level intakes were reported to be as high as 34.5 mg/kg body weight/day in other children, though the highest 'high level' intakes among infants, toddlers, adolescents and adults were reported to range between 28.5 and 31.7 mg/kg body weight/day. In the elderly and very elderly, high level intakes were reported to be up to 18.6 and 13.0 mg/kg body weight/day, respectively (see Table IX.B.1-1).

When examined on an absolute basis, intakes were higher in the older age groups. Adolescents, adults and the elderly all the had the highest upper range of mean and high level intakes at up to 393, up to 483 and up to 380 mg/day, respectively at the mean, and up to 1,659, up to 2,107 and up to 1,414 mg/day, respectively at the high level.

Table IX.B.1-1 Estimated Daily Intake of Bonolive® from Its Proposed Food Uses Based on the EFSA Comprehensive Database in Different Population Groups

Population Group	No. of Surveys[1]	Body Weight Intakes of Bonolive® (mg/kg bw/day)[2]		Absolute Intakes of Bonolive® (mg/day)[3]	
		Mean Range	High Level Range	Mean Range	High Level Range
Infants (≤ 11 months)	6	0.1 to 4.5	0.6 to 28.5	1 to 23	3 to 143
Toddlers (12 to 35 months)	12	1.1 to 10.8	4.2 to 30.3	13 to 130	50 to 364
Other Children (3 to 9 years)	22	1.1 to 10.8	2.5 to 34.5	25 to 249	58 to 797
Adolescents (10 to 17 years)	18	0.5 to 7.5	1.9 to 31.7	26 to 393	99 to 1,659
Adults (18 to 64 years)	23	0.2 to 6.9	1.3 to 30.1	14 to 483	91 to 2,107
Pregnant and Lactating Women	2	0.4 to 0.6	5.4 to 7.2	28 to 42	378 to 504
Elderly (65 to 74 years)	16	0.1 to 5.0	0.5 to 18.6	8 to 380	38 to 1,414
Very Elderly (≥ 75 years)	13	0.1 to 4.2	0.8 to 13.0	7 to 299	57 to 926

bw = body weight; EFSA = European Food Safety Authority.
[1] Includes only surveys in which consumption data were identified for the proposed food categories in which Bonolive® is used.
[2] Estimated intakes calculated using consumption data from the EFSA Comprehensive database.
[3] Estimated intakes calculated using consumption data on a body weight basis (mg/kg bw/d) multiplied by default body weights for each population group

IX.B.1.2 Main Contributing Categories

Overall, the top contributors to Bonolive® intake among all age groups were the food categories 'Fruit juice' (ranging between 6.4 and 93.7%) and 'Bottled water' (ranging between 5.8 and 92.5%). From infants to adolescents, 'Fruit juice' was the top contributor to Bonolive® intakes, whereas 'Bottled water' was the top contributor from adults to the very elderly. Other food categories which contributed ≥5% to total intakes included 'Muesli' (5.7 to 64.5% among the elderly and the very elderly), 'Yoghurt, cow milk, with fruit' (7.5 to 64.4% among infants and toddlers), 'Jelly candies' (7.2 to 48.1% among adolescents), and 'Chewing gum without added sugar' (5.6 to 48.0% among children and adults).

IX.B.1.3 Summary

As mentioned above, products containing Bonolive® will clearly be targeted towards individuals aged over 50 years. As such, the estimated daily intakes of most relevance are those calculated for adults, elderly and the very elderly; wherein the mean range spans from 7 to 483 mg/day (equivalent to 0.1 to 6.9 mg/kg body weight/day), and the high level range spans from 57 to 2,107 mg/day (0.5 to 30.1 mg/kg body weight/day) (see Table IX.B.1-1). Considering the average "maximum" use level of Bonolive®, of 179 mg/serving[11], the intakes by these population groups are equivalent to between <1 to 3 servings/day[12] at the mean and between <1 to 12[13] servings/day at the high level. The upper range of these high level

[11] Range of use levels (mg/serving) of Bonolive (see Table IX.A-1): 25 mg/serving (yoghurts) to 332 mg/serving (gummies). Mean value = 179 mg/serving
[12] Calculation = (Mean intakes: 7 to 483 mg/day) / (179 mg/serving) = 0.04 to 2.7 servings/day
[13] Calculation = (High level intakes: 38 to 2,107 mg/day) / (179 mg/serving) = 0.2 to 11.8 servings/day

intakes were considered to be unrealistic (*i.e.*, that an individual would chronically consume 12 servings per day of products containing Bonolive®), as such, it was deemed necessary to conduct a more refined assessment.

IX.B.2 UK NDNS Dataset

IX.B.2.1 Estimated Daily Intakes

Estimates for the total daily intakes of Bonolive® from all proposed food-uses are provided in Tables IX.B.2-1 and IX.B.2-2, on an absolute (mg/person/day) and body weight (mg/kg body weight) basis, respectively.

As would be expected for a 4-day survey, the percentage of users was high among all age groups evaluated in the current intake assessment; greater than 57% of the population groups consisted of users of those food products in which Bonolive® is currently proposed for use (Table IX.B.2-1). Toddlers and children had the greatest percentage of users at 91.9%. As consumers of Bonolive® represent the population of interest from a risk assessment vantage, only the consumer-only intake results will be discussed in detail.

Mean and 95th percentile intakes of Bonolive® by the total population were estimated to be 64 and 221 mg/person/day. Adolescents were determined to have the greatest mean and 95th percentile consumer-only intakes of Bonolive® on an absolute basis, at 109 and 333 mg/person/day, respectively. When focusing on the target population (*i.e.,* ages 50 years and older), the exposure levels were estimated to be 41 and 131 mg/person/day at the mean and 95th percentile, respectively (Table IX.B.2-1).

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

Population Group	Age Group (Years)	n	Total Population Intake (mg/day)		Consumer-Only Intake (mg/day)			
			Mean	95th Percentile	%	n	Mean	95th Percentile
Toddlers	1<3	238	36	113	91.9	218	39	116
Children	3 to 9	844	67	215	91.9	771	73	216
Teenagers	10 to 17	900	89	305	81.1	731	109	333
Adults	18 to 64	1,746	43	182	68.3	1,171	62	224
Elderly	65 to 74	247	25	82	69.3	163	36	95
Very elderly	≥75	181	20	78	57.0	101	35	91*
Target Population	***≥50***	***972***	***27***	***101***	***65.8***	***625***	***41***	***131***
Total	All ages	4,156	46	188	71.2	3,155	64	221

NDNS = National Diet and Nutrition Survey; UK = United Kingdom.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

On a body weight basis, the consumer-only intakes of Bonolive® by the total population were 1.2 and 4.7 mg/kg body weight/day at the mean and 95th percentile, respectively.

Children were identified as having the highest mean and 95th percentile consumer-only intakes of any population group, of 3.2 and 9.3 mg/kg body weight/day, respectively. The consumer-only intakes of Bonolive® were 0.5 and 1.7 mg/kg body weight/day at the mean and 95th percentile, respectively, for the target population (≥50 years), see Table IX.B.2-2.

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

Population Group	Age Group (Years)	n	Total Population Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
			Mean	95th Percentile	%	n	Mean	95th Percentile
Toddlers	1<3	209	2.8	8.6	92.3	192	3.0	8.9
Children	3 to 9	799	2.9	9.0	92.1	733	3.2	9.3
Teenagers	10 to 17	867	1.8	5.8	80.8	703	2.2	6.9
Adults	18 to 64	1,634	0.6	2.4	68.7	1,100	0.8	3.1
Elderly	65 to 74	231	0.3	1.1*	70.2	154	0.5	1.3*
Vey Elderly	≥75	162	0.3	1.1*	57.6	92	0.5	1.5*
Target Population	***≥50***	***899***	***0.4***	***1.4***	***66.5***	***583***	***0.5***	***1.7***
Total Population	All ages	3,902	0.9	3.9	71.7	2,974	1.2	4.7

bw = body weight; NDNS = National Diet and Nutrition Survey; UK = United Kingdom.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

IX.B.2.2 Main Contributing Categories

The total UK population was identified as being abundant consumers of flavoured yoghurts (31.5 to 79.6% of users) and fruit juices (37.1 to 60.3% of users). In terms of contribution to total mean intake of Bonolive®, fruit juices (contributed 18.3 to 50.4% to total intakes) was the main source of intake across all population groups, followed by cereal and granola bars (5.7 to 20.7%) and flavoured yogurts (4.2 to 28.0%). Nutrition bars and vegetable juices individually contributed <1% to total mean Bonolive® intakes across all population groups.

IX.B.2.3 Summary

The mean and 95th percentile intakes of Bonolive® by the target population in the UK (ages 50 years and older) were calculated at 41 and 131 mg/person/day, respectively (equivalent to 0.5 and 1.7 mg/kg body weight/day). The average "maximum" use level for fruit juices and yoghurts (the highest contributing categories to total Bonolive® intakes in terms of both percent consumers and contribution to mean intakes), is 32.5 mg/serving[14]. As such, the

[14] Range of use levels (mg/serving) of Bonolive (see Table IX.A-1): 25 mg/serving (yoghurts) to 40 mg/serving (yoghurts). Mean value = 32.5 mg/serving

mean and 95th percentile intake values in this age group are equivalent to just over 1 serving/day[15] and 4 servings/day[16], respectively.

IX.B.3 Combined Intakes from Food Supplements

As specified in Table IX.A-1, Bonolive® is also intended for use in food supplements at a maximum dose of 250 mg/day. These products would primarily be targeted towards individuals aged 50 years and over. A cumulative assessment of intakes has been determined by summing the maximum intended daily dose of Bonolive® in food supplements (250 mg/day) with the estimated mean and heavy level (95th percentile) intakes from food and beverages by the target population, as calculated using the UK NDNS dataset. This dataset was used as the EFSA Comprehensive only allows calculation for pre-determined population groups, whereas the results could be adapted to select only intakes by this cohort of interest.

The estimated mean and 95th percentile intakes of the target population were estimated at 41 and 131 mg/person/day, respectively (equivalent to 0.5 and 1.7 mg/kg body weight/day). These figures were used to determine the cumulative exposure from foods and beverages containing this ingredient, as well as food supplements. The resulting mean intakes are calculated at 291 mg/person/day or 4.1 mg/kg body weight/day for a 70 kg adult[17,18] (EFSA, 2012); the 95th percentile intakes were determined to be 381 mg/person/day or 5.3[19] mg/kg body weight/day. There is a low probability that an individual will be among the top 5% of consumers of food products containing Bonolive® (*i.e.*, consumer with 95th percentile intakes) *and* a consumer of supplements. As such, 291 mg/person/day (or 4.1 mg/kg body weight/day) are considered more realistic for chronic estimates of intake for this ingredient. However, for the sake of completeness all figures are included herein.

IX.B.4 Estimated Daily Intakes from Foods for Special Medical Purposes

The maximum intended use level in foods for special medical purposes is 625 mg/day. These products would be targeted only towards adults, and it is anticipated that Bonolive® would be the only source of olive leaf extract in the diet of these individuals; as such, for a 70 kg adult (EFSA, 2012), the corresponding intakes would be 8.9 mg/kg body weight/day.

IX.B.5 Summary

The intakes of Bonolive® from its proposed uses in the EU have been estimated using 2 sources of consumption data in the EU. As per the recommended approach, the EFSA

[15] Calculation = (Mean intake: 40 mg/day) / (32.5 mg/serving) = 1.2 servings/day
[16] Calculation = (P95 intake: 131 mg/day) / (32.5 mg/serving) = 4.0 servings/day
[17] Calculation: [(250 mg/day) / (70 kg)] + 0.5 mg/kg body weight/day
[18] The average body weight by the target population was calculated at 82 kg [(Bonolive® intakes on an absolute basis) / (Bonolive® intakes on a body weight basis)]. This demonstrates good agreement with the default body weight, but also illustrates that the default body weight utilised here was conservative, resulting in a higher estimate of exposure when expressed on a body weight basis.
[19] Calculation: [(250 mg/day) / (70 kg)] + 1.7 mg/kg body weight/day

Comprehensive database was initially used as a screening estimate of intakes (or Tier 1). Intakes were subsequently refined using individual-based data from the UK NDNS 2008-2012 dataset (Tier 2). All results, along with a summary of the main uncertainties associated with both assessment models, are summarised in Table IX.B.5-1, and the main findings are summarised below.

Summary statistics for FoodEx Level 2 and Level 3 food categories from the EFSA Comprehensive Database were used to estimate the mean and high level intakes of Bonolive® in specific demographic groups in different Member States in the EU. The availability of summary statistics alone for determining total mean and high level intakes does not take into account individual consumption patterns, and results in the assumption that the ingredient will be added to an entire food category, as opposed to specific uses which are deemed suitable based on the composition/food matrix. Furthermore, the method for calculation of the 'high level' intakes involves summing intakes by food category consumers only which may or may not be mutually exclusive. Nonetheless, using this dataset, the highest intakes of Bonolive® on a body weight basis were observed in toddlers and other children. Toddlers and other children had the highest estimated mean intake values, both with a range of 1.1 to 10.8 mg/kg body weight/day, and other children were calculated to have highest 'high level' intakes with a range of 7.7 to 114.9 mg/kg body weight/day. As mentioned above, children, and in particular toddlers, often consume the highest amount of food when considered on a body weight basis when compared to the rest of the population (EFSA, 2011a). Further, products containing the ingredient will clearly be targeted towards individuals aged over 50 years. As such, intakes described for adults, elderly, and very elderly population groups, wherein the mean intakes ranged from 7 to 483 mg/day (0.1 to 6.9 mg/kg body weight/day) and the high level intakes ranged from 38 to 2,107 mg/day (0.5 to 30.1 mg/kg body weight/day), are expected to be more accurate estimates of chronic dietary exposure within the intended target population. As mentioned above, the EFSA Comprehensive assessment is used to provide a screening estimate of intakes. Considering the average "maximum" use level of Bonolive®, of 179 mg/serving[20], these intakes are equivalent to between <1 to 3 servings/day[21] at the mean and between <1 to 12[22] servings/day at the high level. The upper range of these high level intakes were considered to be unrealistic (*i.e.*, that an individual would chronically consume 12 servings per day of products containing Bonolive®), as such, it was deemed necessary to conduct a more refined assessment.

When more refined estimates of intakes were examined using individual food consumption data from the UK NDNS dataset, teenagers were estimated to have the highest mean and 95th percentile intakes on an absolute basis of 109 mg/person/day (2.2 mg/kg body weight/day) and 333 mg/person/day (6.9 mg/kg body weight/day), respectively. Children had the highest intakes of Bonolive® on a per body weight basis with the highest mean and

[20] Range of use levels (mg/serving) of Bonolive (see Table IX.A-1): 25 mg/serving (yoghurts) to 332 mg/serving (gummies). Mean value = 179 mg/serving
[21] Calculation = ((Mean intakes: 7 to 483 mg/day) / (179 mg/serving) = 0.04 to 2.7 servings/day
[22] Calculation = ((High level intakes: 38 to 2,107 mg/day) / (179 mg/serving) = 0.2 to 11.8 servings/day

95th percentile consumer only intakes of 3.2 mg/kg body weight/day and 9.3 mg/kg body weight/day, respectively. However, as mentioned above, these are not the target population groups and are presented for completeness. When mean and 95th percentile intakes of Bonolive® by the target population in the UK (ages 50 years and older) were examined, they were calculated at 41 and 131 mg/person/day, respectively (equivalent to 0.5 and 1.7 mg/kg body weight/day). As mentioned in Section IX.B.1, fruit juices and yoghurts were the top contributing categories among all age groups in terms of percent consumers and contribution to mean intakes. Considering the average "maximum" use level for these two categories of 32.5 mg/serving[23], the intake values in this age group are equivalent to just over 1 serving/day[24] at the mean, and 4 servings/day[25] at the 95th percentile.

Cumulative exposure from foods and beverages, as well as food supplements containing Bonolive® has been determined using the estimates for individuals aged over 50 years (*i.e.*, the target population) from the UK NDNS dataset, as well as the maximum intended dose in food supplements (250 mg/day). The resulting mean and 95th percentile intakes were calculated at 291 and 381 mg/person/day, respectively; this is equivalent to 4.1 and 5.3 mg/kg body weight/day for a 70 kg adult (EFSA, 2012).

Finally, Bonolive® is also proposed for use in foods for special medical purposes at a dose of 625 mg/day. The maximum intended use level in foods for special medical purposes is 625 mg/day. These products would be targeted only towards adults, and the daily intake of Bonolive® would be limited to only these products in the diet; as such, for a 70 kg adult (EFSA, 2012), the corresponding intakes would be 8.9 mg/kg body weight/day.

[23] Range of use levels (mg/serving) of Bonolive (see Table IX.A-1): 25 mg/serving (yoghurts) to 40 mg/serving (yoghurts). Mean value = 32.5 mg/serving
[24] Calculation = (Mean intake: 40 mg/day) / (32.5 mg/serving) = 1.2 servings/day
[25] Calculation = (P95 intake: 131 mg/day) / (32.5 mg/serving) = 4.0 servings/day

Table IX.B.5-1 Summary Figures for Bonolive® Intakes Calculated Using the EFSA Comprehensive Database and UK NDNS

Dataset/Approach	Uncertainty Analysis (Direction)	Population Group (Age)	Absolute Intakes (mg/day)[1,2]		Body Weight Intakes (mg/kg bw/day)[2]	
			Mean	High Level	Mean	High Level
EFSA Comprehensive – Deterministic Model Screening Tool; Includes data for 16 EU Member States	Uses summary statistics for individual food groups for survey; 'High level' intakes calculated by summing top contributing P95 intakes for individual food categories to mean intakes of all other food categories (++) Assumes all foods within a FoodEx food category contain the ingredient at the maximum identified use level (++) Absolute intakes presented in this table have been calculated using default body weight (+/-) Different methodologies / sampling error or bias / mis- or under-reporting of food groups / no standard portion size across member states (+/-)	Infants (≤11 m)	1 to 23	3 to 143	0.1 to 4.5	0.6 to 28.5
		Toddlers (12-35 m)	13 to 130	50 to 364	1.1 to 10.8	4.2 to 30.3
		Other Children (3-9 y) years)	25 to 249	58 to 797	1.1 to 10.8	2.5 to 34.5
		Adolescents (10-17 y)	26 to 393	99 to 1,659	0.5 to 7.5	1.9 to 31.7
		Adults (18-64 y)	14 to 483	91 to 2,107	0.2 to 6.9	1.3 to 30.1
		Pregnant & Lactating Women	28 to 42	378 to 504	0.4 to 0.6	5.4 to 7.2
		Elderly (65-74 y)	8 to 380	38 to 1,414	0.1 to 5.0	0.5 to 18.6
		Very Elderly (≥75 y)	7 to 299	57 to 926	0.1 to 4.2	0.8 to 13.0
UK NDNS Rolling Survey Years 1-4 – Distributional More Realistic Assessment; Uses on Individual-Based Food Consumption Data	Assumes foods which fit within the identified food categories contain the ingredient at the maximum intended use level (+) Extrapolation of food consumption from UK to all other EU Member States (++/- -) Mis-/under-reporting for some food groups of interest, *e.g.* snacks (-) Use of a 4-day diary (+) Values for cumulative exposure assumes 'users' of foods containing ingredient are also consuming food supplements containing this ingredient daily	Toddlers (1<3 y)	39	116	3.0	8.9
		Children (3-9 y)	73	216	3.2	9.3
		Adolescents (10-17 y)	109	333	2.2	6.9
		Adults (18-64 y)	62	224	0.8	3.1
		Elderly (65-74 y)	36	95	0.5	1.3
		Very elderly (≥75 y)	35	91	0.5	1.5
		Target population (≥50 y)	***41***	***131***	***0.5***	***1.7***
		Target population (≥50 y) + Supplements[3]	***291***	***381***	***4.1***	***5.3***
		Total population (≥1.5y)	64	221	1.2	4.7

bw = body weight; EFSA = European Food and Safety Association; m = month; UK NDNS = United Kingdom National Diet and Nutrition Survey; y = year.
Figures ***bolded and italicised*** are the intake values calculated for the target population.
[1] Absolute intakes for EFSA Comprehensive calculated by multiplying body weight intakes by EFSA default (or mean) body weights for respective age categories (EFSA, 2012), *i.e.*, infants = 5 kg; toddlers = 12 kg; other children = 23.1 kg; adolescents = 52.35 kg; adults = 70 kg; elderly = 76 kg; very elderly = 71.2 kg (no values available for pregnant or lactating women).
[2] Consumer-Only intakes presented for NDNS
[3] Values calculated as: [Intakes determined for 'Total population ≥50 years'] + [250 mg/day (or 3.6 mg/kg body weight/day for a 70 kg adult)].

IX.C Geographical Restrictions

The marketing of food and food supplements containing Bonolive® will not be restricted geographically.

IX.D Will the Novel Food Replace Other Foods

Bonolive® is not intended to replace other foods currently on the market.

X INFORMATION FROM PREVIOUS HUMAN EXPOSURE TO Bonolive®

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

- “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.B.

X.A Information from Previous Human Exposure

As demonstrated in Section III.D, olive leaves and polyphenols from olives/olive leaves have been consumed traditionally for health purposes. Several olive leaf derived products including several dried, aqueous and/or less concentrated extractions of olive leaf oils, powders, and extracts are on the market in the EU as food supplements.

Black olives typically contain between 115 and 214 mg oleuropein/100 g fresh olives (Bianchi, 2003). With a typical olive (no stone, stuffed) weighing approximately 3 g (FSA, 2002), and assuming that a typical handful (serving) would be 5 to 6 olives, this corresponds to approximately 38.5 mg oleuropein per serving of fresh olives. Individuals who consume the typical Mediterranean diet generally ingest up to 90 mg (± 105 mg) of polyphenols from olives per day (combined intake from olive oil [21.9 ± 10.4 mg] and olives [68.5 ± 104 mg]) (de Bock *et al*., 2013a,b; Tresserra-Rimbau *et al*., 2013). As determined in Section IX above, when focusing on the target population (*i.e*., ages 50 years and older), the exposure levels of Bonolive® were estimated to be 41 and 131 mg/person/day at the mean and 95th percentile, respectively. Considering that oleuropein is present in Bonolive® at 40%, this corresponds to an intake of 16.4 and 52.4 mg/person/day, at the mean and 95th percentile, which is not significantly different from typical intakes of oleuropein from olives.

Bonolive® received self-Generally Recognized as Safe (GRAS) status in the United States (U.S.) in March 2016 and has been on the market in foods in the U.S. since that time. Bonolive® also has been on the U.S. market as a dietary supplement since 2013. A natural health product containing Bonolive® has been licensed in Canada, which includes health claims approved by Health Canada. Bonolive is approved as an ingredient in a complimentary medicine by the Therapeutic Goods Association (TGA) in Australia. Bonolive® also is included in supplements in Japan, Malaysia, Taiwan and India.

X.B Potential Allergenicity Concerns

Bonolive® does not contain any of the allergens listed in Regulation (EC) 1169/2011[26]. Allergic reactions to pollen from olive trees have been reported frequently in the literature, occurring mainly in Mediterranean areas where *Olea europaea* L. trees are commonly found. Sensitive individuals may suffer symptoms of allergic rhinitis, conjunctivitis and asthma as a result of inhalation exposure (Moreno *et al*., 2015). It is important to note that, as described in Section II.A.1, the leaves used to manufacture Bonolive® are obtained in February, prior to blossoming in May. As such, exposure to pollen is limited. Furthermore, the manufacturing process for Bonolive® includes several purification steps.

[26] Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004. OJ L 304, 22.11.2011, p. 18-63.

Contact (topical) allergy to olive oil is rare, and may result in eczema-type symptoms in sensitive subjects, although ingestion of the oil may often still be tolerated (Isaksson and Bruze, 1999; Williams and Tate, 2006). Despite its common consumption, food allergy reactions to olive fruits is extremely rare, although it has been reported (Unsel *et al*., 2009). Reports of allergic reactions to olive leaves, olive leaf extracts or oleuropein were not identified.

XI NUTRITIONAL INFORMATION ON Bonolive®

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

- “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 collectively in Sections XI.A and XI.C.

XI.A Nutritional Equivalence to Existing Foods

Bonolive® is not nutritionally equivalent to other foods and is not intended to replace other foods currently on the market in the EU other than to provide an alternative source of olive polyphenols from the "Mediterranean diet".

XI.B Other Nutritional Considerations

A summary of the nutritional composition of Bonolive® is presented below in Table XI.B-1. Based on the results, Bonolive® is not anticipated to negatively impact the quality of the diet.

The nutritional composition of Bonolive® is presented below in Table XI.B-1

Table XI.B-1 Typical Nutritional Composition of Bonolive®

Component	**Percentage**
Energy	386 kcal/100 g 1638 KJ/100 g
Total Fat	0.2%
Mono-unsaturated fat	0.1%
Poly-unsaturated fat	0.04%
Carbohydrates (including polyphenols)	94.8%
Fibre	<0.1%
Ash	1.67%
Humidity	2.2%
Protein	1.16%
Sodium	0.172%
Sugars	<1%

XI.C Nutritional Benefit

Bonolive® is a source of oleuropeins (polyphenols), with a content of at least 40%. Oleuropein is associated with many health benefits including antioxidant, antihypertensive, hypercholesterolemic, and cardioprotective activities (Vogel *et al*., 2015). Studies also have evaluated the anti-inflammatory effects of oleuropein in trauma of bone marrow. The clinical trial by Filip *et al.* (2015), discussed in Section XIII.C.1, in which 64 osteopenic subjects consumed Bonolive® or a placebo for 12-month also included analysis of its beneficial effects related to bone health. It was determined in this study that bone mineral density remained stable in the treatment group while a decrease was observed in the placebo group and that Bonolive® positively affects serum osteocalcin levels and improves serum lipid profiles (lower LDL cholesterol and improved HDL/total cholesterol ratio).

One health claim for olive oil polyphenols, including oleuropein, related to the protection of blood lipids from oxidative stress has been authorised according to Regulation (EC) No

1924/2006[27] on nutrition and health claims (EFSA, 2011b). Currently, however, there are no authorised health claims according to Regulation (EC) No 1924/2006 on nutrition and health claims for oleuropein/polyphenols and bone health specifically and thus, a health claim application pursuant to this regulation will be submitted for Bonolive® and bone health in older individuals prior to the use of any such a claim on products containing this ingredient.

[27] Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. OJ L 404, 30.12.2006, p. 9–25.

XII MICROBIOLOGICAL INFORMATION ON Bonolive®

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

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

This question has been addressed in Section XII.A.

XII.A Microbiological Specifications and Analyses for Bonolive®

The microbiological specifications and batch analyses are presented above in Table I.C-1. The results confirm that the production process does not introduce a potential for microbiological contamination.

XIII TOXICOLOGICAL INFORMATION ON Bonolive®

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

- "Is there a traditional counterpart to the novel food that can be used as a baseline to facilitate the toxicological assessment?"
- "Compared to the traditional counterpart, does the novel food contain any new toxicants or changed levels of existing toxicants?"

or

- "Is there information from a range of toxicological studies appropriate to the novel food to show that the novel food is safe under anticipated conditions of preparation and use?"
- "Is there information which suggests that the novel food might pose an allergenic risk to humans?"

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

XIII.A Absorption, Distribution, Metabolism, and Elimination

The absorption, distribution, metabolism, and renal clearance of phenolic compounds from olive leaves and their extracts is considered to be a relatively rapid process (Japon-Lujan *et al.*, 2006; de Bock *et al.*, 2013a,b). Following oral ingestion, the phenolic compounds and secoiridoid derivatives from olive leaf extracts are hydrolysed in the upper gastrointestinal tract and absorbed from the small intestine (Vissers *et al.*, 2002; Corona *et al.,* 2006; Suarez *et al.,* 2010; García-Villalba *et al.*, 2014). The mechanism of absorption of olive oil phenolics has yet to be fully elucidated, although passive diffusion, transcellular, paracellular or glucose transporter mechanisms have been proposed, and the polarities of the phenolics have also been suggested to play a role (Cicerale *et al.*, 2009; García-Villalba *et al.*, 2014).

The absorption of olive oil polyphenols following ingestion has been investigated in human subjects (Vissers *et al.*, 2002). Since phenols are known to be degraded by microorganisms in the colon, which can lead to overestimation of absorption in the analysis of faecal excretion, 8 otherwise healthy subjects with an ileostomy were chosen for the study, along with 12 healthy subjects with a colon, for comparative purposes. The study subjects consumed 3 different supplements containing 100 mg of olive oil phenols with breakfast on separate days, separated by a 1-week washout period. Ileostomy subjects consumed a supplement containing: (i) mainly nonpolar phenols (*e.g.* oleuropein- and ligstrosideaglycones; as an ethanolic extract of olive oil), (ii) mainly polar phenols (*e.g.* hydroxytyrosol and tyrosol; as a reverse osmosis extract of olive oil), and (iii) oleuropein-glycoside (commercially available from Solgar Laboratories). Subjects with a colon consumed the same supplements as the ileostomy subjects, except that a supplement without phenols (placebo) was given instead of the supplement with oleuropein-glycoside. Intake of olives or olive oil was not permitted during the study. Ileostomy effluent and urine were collected for 24 hours after supplement intake. Phenol concentrations were measured using high performance liquid chromatography (HPLC). The concentrations of tyrosol and hydroxytyrosol detected in the ileostomy effluent were considered to be low (<4%), and no aglycones were detected. Absorption of the compound was confirmed by the excretion of approximately 5 to 6% of tyrosol and hydroxytyrosol in urine from both subject groups that consumed the polar supplement, 6 to 12% of tyrosol and hydroxytyrosol after consuming the nonpolar supplement, and ileostomy subjects excreted 16% of these compounds (mainly as hydroxytyrosol) after consuming the oleuropeinglycoside supplement. Oleuropein and ligstroside-aglycones were not measured. Based on this data, the authors estimated that up to 66% of the phenols from the nonpolar supplement were absorbed in the small intestine, and this percentage would be higher for the polar supplement and the oleuropein-glycoside.

While numerous metabolites (>80) of olive polyphenols have been identified in plasma and urine following their ingestion, the majority of metabolites tend to be identified in their conjugated forms (primarily sulfonated and glucuronidated), suggesting extensive first-pass intestinal/hepatic metabolism of these compounds prior to excretion in the urine (Miro-Casa *et al.*, 2003; García-Villalba *et al.*, 2010, 2014; Suarez *et al.*, 2010; Deiana *et al.*, 2011). Colonic microflora are also likely to play a role in the biotransformation of these compounds

(Corona *et al.*, 2006). The bioavailability and metabolic profile of the olive leaf extract Bonolive® was investigated in healthy pre- and post-menopausal women. Whilst the full study report is confidential, the study has been summarised in a publication (García-Villalba *et al.*, 2014). Following the oral ingestion of 250 mg of Bonolive® or placebo, metabolites in 24-hour plasma and urine samples were analysed using high performance liquid chromatography coupled to electrospray ionisation-quadruple time of flight mass spectrometry (HPLC-ESI-QTOF) and ultra-performance liquid chromatography tied to electrospray triple quadrupole mass spectrometry (UPLC-ESI-QqQ). Using targeted analysis, a total of 47 metabolites that could be derived from the intake of olive leaf extract were searched for. None of the parent compounds present in the olive leaf extract were found in the plasma or urine. Almost all of the identified metabolites were in conjugated form, mainly glucuronidated and sulphated. The major metabolites found in plasma were 3 metabolites derived from hydroxytyrosol, 4 oleuropein aglycon derivatives, and 2 homovanillic acid metabolites. These metabolites appeared rapidly in the plasma, with the maximum peak concentration occurring within the first 35 to 75 minutes. The urinary metabolite profile was very similar to that found in plasma, with the exception of homovanillic alcohol sulphate, elenoic acid and elenoic glucuronide, which appeared exclusively in urine, and the absence of luteolin. Maximum urine excretion rate was reached in the first 4 hours, followed rapid decrease toward baseline levels. The exception was for the sulphated metabolites, the excretion of which was not complete by 24 hours (the time limit of the study). The first metabolite to reach the maximum peak concentration was hydroxytyrosol glucuronide in both plasma and urine. The absorption patterns of the different phenolic compound in plasma and urine were similar between pre- and post-menopausal women. Plasma levels of hydroxytyrosol glucuronide, hydroxytyrosol sulphate, oleuropein aglycon glucuronide, and oleuropein aglycon derivative 1 were higher in post-menopausal women ($p < 0.05$), and these women also excreted less sulphated metabolites compared to pre-menopausal women. Age and/or hormonal related changes themselves and in relation to gastric emptying and expression of phase II enzymes were suggested as possible reasons for the differences between these groups.

In a similar metabolism study, the bioavailability of oleuropein and hydroxytyrosol from another olive leaf extract was investigated in 9 healthy volunteers (4 females, 5 males) (de Bock *et al.*, 2013b). The subjects were given a single low dose (containing 51.1 mg oleuropein, 5.4 to 9.7 mg hydroxytyrosol) and high dose (containing 76.6 mg oleuropein, 8.1 to 14.5 mg hydroxytyrosol) of the extract in either capsule or liquid form, in a cross-over fashion with a one-week washout period between treatments. Phenolic content in plasma and urine over 24 hours was analysed using Liquid Chromatography-Electrospray Ionization-Mass Spectrometry/Mass Spectrometry (LC-ESI-MS/MS). The primary metabolites identified were the conjugated metabolites of hydroxytyrosol (sulphated and glucuronidated), comprising 96 to 99% of the phenolic metabolites detected in plasma. They were also the primary metabolites found in urine. Oleuropein and hydroxytyrosol metabolites were rapidly detected in plasma after ingestion (within 23 to 93 minutes). Peak oleuropein concentrations in plasma were notably 6-fold higher following ingestion of liquid *versus* capsule preparations. Male subjects displayed greater plasma area-under-the-curve for conjugated

hydroxytyrosol. The majority of metabolite recovery occurred within 8 hours of ingestion. There was marked inter-individual variation in the results, which was reported to be possibly due to differences in human enzymatic activity.

Further, the distribution of olive phenolic compounds following their absorption has been studied in Wistar rats administered a single dose of olive cake, the main by-product of olive oil extraction (Serra *et al.*, 2012). Levels of metabolites (as detected by UPLC-MS/MS) were highest in the liver and kidneys, followed by the testes. The heart, brain, spleen and thymus showed a lower number of metabolites with phenolic acids being the main metabolites quantified, and there was also some evidence suggesting that these metabolites cross the blood brain barrier. The type of phenolic compounds and their metabolites detected (sulphated and glucuronidated conjugates) were similar to those detected in humans.

XIII.B Preclinical Toxicological Studies

A battery of toxicological tests has been conducted to assess the preclinical safety of Bonolive®, including a 14-day dose range-finding oral toxicity study, a 90-day oral toxicity study, a bacterial reverse mutation test, an *in vitro* chromosomal aberration test, and an *in vivo* mammalian erythrocyte micronucleus test. All tests were conducted in accordance with Good Laboratory Practice (GLP) and the Organisation for Economic Co-operation and Development (OECD) Guidelines for the Testing of Chemicals. The details and results of these studies are summarised below in Sections XIII.B.1 to XIII.B.2. In addition, a number of toxicological studies conducted on various olive leaf, fruit, and pulp extracts other than Bonolive® have been identified in the scientific literature. A summary of the details and results of these non-product-specific studies are summarised in Section XIII.B.3 to corroborate the preclinical safety of Bonolive®.

XIII.B.1 Repeat-Dose Toxicity Studies Conducted with Bonolive®

XIII.B.1.1 14-Day Oral Toxicity Study

A 14-day repeat-dose oral toxicity study was conducted to investigate the toxic potential and maximum tolerated dose (*i.e.*, range-finding) of Bonolive® in SPF Crl:(WI)BR Wistar rats. The details of this study are confidential; however, the study was conducted in accordance with OECD Test No. 407 (OECD, 2008) (Clewell *et al*., 2016). Based on the results of this 14-day study, the NOAELs for male and female rats were determined to be 600 and 2,000 mg/kg body weight/day, respectively.

XIII.B.1.2 90-Day Oral Toxicity Study

Based on the results of the 14-day repeat-dose study, the subchronic toxicity of Bonolive® was further evaluated in a 90-day repeat-dose oral toxicity study conducted in rats. Whilst the full study report is confidential, the study has been summarised in a publication (Clewell *et al*., 2016). The study was conducted in accordance with OECD Test No. 408 (OECD, 1998) and U.S. FDA Redbook 2000, IV.C.4.a (U.S. FDA, 2003). Following a 7-day

acclimatisation period, 4 groups of SPF Crl:(WI)BR Wistar rats (10/sex/group) were administered Bonolive® *via* oral gavage at dose levels of 0 (vehicle control), 360 (low-dose), 600 (mid-dose), or 1,000 (high-dose)[28] mg/kg body weight/day for 90 days. The vehicle consisted of a 1% Tween 80 solution prepared in distilled water. In addition, to affirm the significance and repeatability of the hyaline-like droplet findings in the 14-day study, a 28-day satellite group (5 rats/sex/group) was added to the abovementioned dose groups in the 90-day study for early histopathological examination with a specific focus on nephropathy. The continuation of the 90-day study plan was dependent on the findings of the 28-day satellite group.

All animals were observed for mortality twice daily during the course of the study. General clinical observations were performed daily post-administration. Detailed clinical observations were made on all animals weekly. A functional observational battery (FOB) was conducted during the last week of test article administration. Body weight was recorded twice weekly during weeks 1 to 4 and once weekly thereafter. Food consumption was determined weekly to coincide with body weight measurements. Ophthalmologic examinations were performed on all animals before the first administration and on animals of the control and high-dose groups during the last week of administration. Clinical pathology and gross pathology examinations were conducted on all animals the day after the last administration [*i.e.*, on day 28 for animals in satellite group, and on day 90 (males) and day 91 (females) for animals in the main group]. Selected organs (liver, kidneys, testes, epididymides, uterus, fallopian tubes, thymus, spleen, brain, heart, adrenals, ovaries, and thyroid/parathyroid) were weighed. Full histopathological examinations were performed on all animals of the control and high-dose groups. In addition, the kidneys were examined histologically in male satellite animals at all dose groups (control, low-dose, mid-dose, and high-dose). Organs with any other macroscopic findings in the low- and mid-dose groups were processed and examined histologically.

One (1) male and 1 female in the high-dose group died during the study on day 60 and day 2, respectively. In the male animal, death was preceded by salivation, convulsion, prone positioning, decreased activity, dyspnoea and narrow eye aperture, all of which occurred shortly after treatment. Gross pathology examinations revealed dark red lungs, dark red liver, dark colour of the right lobe of the thymus and yellowish fluid content in the thoracic cavity, which is in full compliance with histopathological findings of acute alveolar emphysema accompanied by acute haemorrhage in the lungs and congestion of the liver and thymus. In the female animal that died[29], there were no preceding clinical signs or gross pathological observations, and histopathological examination revealed acute catarrhal pneumonia and serous-fibrinous pleuritis. Both deaths were considered to be incidental (due to the gavage procedure) and not considered to be an adverse effect of the test article. All other clinical signs of toxicity observed in the main test animals were sporadic and occurred with similar frequency in the article and control groups; thus, they were also not

[28] One additional female animal was added to the study group on Day 2 to replace a female animal that died in the high-dose group.
[29] Another female animal was added to this study to replace the female rat that died.

considered to be toxicologically-relevant. In addition, no mortalities or toxicologically-relevant clinical findings were noted in the 28-day satellite group animals.

In the main 90-day study, transient lower mean daily body weight was noted in high-dose females between days 11 to 14, 28 to 35, and 56 to 63; however, no significant differences in end body weight or in total body weight gain were observed compared to control animals. Thus, these changes were noted to be indicative of biological variation and were not considered to be biologically or toxicologically relevant. Mean daily food consumption was not affected by the administration of the test article. Several sporadic statistically significant differences in feed efficiency were observed, including slightly lower feed efficiency in low- and high-dose males between days 28 and 35 and in mid-dose females between days 0 and 7; however, these observations were all noted to be within normal biological variation. There were no test article-related ophthalmologic abnormalities noted in any test animals. In addition, no test article-related effects on animal behaviour or neurological functioning were noted in the FOB conducted for both the main or satellite animals.

In the main 90-day study, the haematological observations included: a statistically significant increase in monocytes in low-dose males; a statistically significant increase in platelets in low-dose females; and, a statistically significant increase in activated partial thromboplastin time (APTT) in mid-dose females. Statistically significant haematological observations in the satellite animals included: increases in neutrophils and decreases in lymphocytes in low- and mid-dose males; an increase in monocytes in mid-dose males; decreases in red blood cells, haemoglobin, and haematocrit in low-dose males; and, a decrease in monocytes in mid-dose females. Since these effects were observed without any dose-dependency, they were considered to be incidental observations that were not attributed to an effect of the test article. Upon clinical chemistry analyses, the following statistically significant changes were observed in the main test animals: a decrease in ALT in mid- and high-dose males and in females of all dose groups; a decrease in AST in high-dose males and females; a decrease in ALP in mid- and high-dose males; a decrease in total bilirubin in low-dose males; a decrease in creatinine in mid- and high-dose males; a decrease in bile acids in low-dose males and an increase in bile acids in low- and high-dose females; an increase in inorganic phosphorus in mid-dose males an increase in calcium in mid- and high-dose males and a decrease in calcium in mid- and high-dose females; a decrease in sodium in mid- and high-dose males; an increase in chloride in low-dose males and a decrease in chloride in high-dose females; and an increase in albumin in high-dose males. Statistically significant clinical chemistry observations in the satellite animals included: a decrease in ALT in low-dose males and mid- and high-dose females; decreases in ALP and cholesterol in mid- and high-dose males; an increase in albumin/globulin ratio in low- and high-dose males; and increases in total bilirubin, sodium, chloride, albumin, and total protein in low-dose females. Given that the above changes in the clinical chemistry parameters were observed with no dose-dependency, the values remained within historical control ranges, and there were no histopathological correlates, these changes were not considered to be of biological or toxicological relevance.

A statistically significant increase in mean relative-to-body liver weight was observed in mid- and high-dose males in the satellite study, as well as in low- and high-dose males and mid-dose females in the main study; however, these changes were all noted to be slight in magnitude and no histopathological observations were detected in the liver. Thus, these observations were not considered to be toxicologically relevant. At necropsy, one-side pyelectasia was observed in a few male animals in the satellite study (2 mid-dose) and main study (3 mid-dose), and hydrometra in the uterus was observed in a few female animals in the satellite study (1 low-dose, 1 mid-dose, and 3 high-dose) and in the main study (1 control, 2 low-dose, 3 mid-dose, and 1 high-dose). Pyelectasia is noted to be a species-specific alteration that occurs commonly in untreated animals of this particular species and strain. In addition, since pyelectasia was observed only in mid-dose animals, and not high-dose animals, it was considered to be toxicologically-relevant finding. Hydrometra in the uterus is noted to be a frequent observation in experimental rats, which is related to the female sexual cycle; thus, it also was not considered to be toxicologically relevant. Additional observations at necropsy also were incidental, non-dose-dependent and/or observed with similar frequency in the control group, and were thus not considered to be toxicologically relevant. Histopathological examinations revealed alveolar emphysema and hyperplasia of bronchus associated lymphoid tissue (BALT) in the lungs of some male and female test animals in the satellite and main study. However, given that these findings were observed with equal frequency in the control and high-dose animals and/or at increased incidences in control animals, they were considered to be incidental and not related to the administration of the test article. In addition, acute pulmonary emphysema and haemorrhages in the thymus were considered to be consequences of hypoxia, dyspnoea and circulatory disturbance developed during exsanguination, and hyperplasia of BALT is noted to be an immunological phenomenon that is not considered to be of toxicological significance. Additional histopathological observations included haemorrhage in the thymus (1 control male in the satellite study), and dilatation of the uterine horns (3 high-dose females in the satellite study, as well as 1 control and 1 high-dose female in the main study). The dilatation of the uterine horns was not considered to be toxicologically relevant as this is a common neurohormonal phenomenon in connection with the proestrus phase of the sexual cycle.

Thus, based on these results, the administration of Bonolive® did not result in adverse effects in the test animals, both in the main study and the satellite study. In contrast to the results obtained in the 14-day oral toxicity study, no renal changes (*i.e.*, hyaline-like droplets in the epithelial cells of the proximal convoluted tubules) were observed. Therefore, the NOAEL for this study was determined to be 1,000 mg/kg body weight/day, the highest dose tested, for both male and female rats.

XIII.B.2 Mutagenicity and Genotoxicity Studies Conducted with Bonolive®

XIII.B.2.1 Bacterial Reverse Mutation Test

The potential mutagenicity of Bonolive® was evaluated in a bacterial reverse mutation test (Ames test) conducted according to OECD Test No. 471 (OECD, 1997a), Commission

Regulation (EC) No 440/2008[30] B13/14, EPA Health Effects Test Guidelines OPPTS 870.5100 (U.S. EPA, 1998a), and ICH Guidance S2(R1) (ICH, 2011). Whilst the full study report is confidential, the study has been summarised in a publication (Clewell *et al*., 2016). Initially, a preliminary range-finding test was conducted using the plate incorporation method at test article concentrations of 5 to 5,000 µg/plate in *Salmonella typhimurium* TA98 and TA100 in the absence and presence of S9 metabolic activation. Based on negative results obtained in the preliminary test, a main test (plate incorporation method) and a confirmatory test (pre-incubation method) was conducted using test article concentrations of 51.2, 128, 320, 800, 2,000, and 5,000 µg/plate in *Salmonella typhimurium* TA98, TA100, TA1535, and TA1537 and *Escherichia coli* WP2 uvrA in the absence and presence of S9 metabolic activation. The negative control consisted of the vehicle (ultrapure water). Appropriate positive controls were also included (4-nitro-1,2-phenylene-diamine, sodium azide, 9-aminoacridine, and methyl-methanesulfonate in the absence of metabolic activation, and 2-aminoanthracene in the presence of metabolic activation).

No biologically relevant positive responses were observed in the preliminary test, the main test, or the confirmatory test at any concentration tested in the absence or presence of S9 metabolic activation. In contrast, the positive control substances displayed marked mutagenic activity. Based on these results, Bonolive® was considered to be non-mutagenic under the conditions of this study.

XIII.B.2.2 In vitro Mammalian Chromosomal Aberration Test

The clastogenic potential of Bonolive® was investigated in a chromosomal aberration test conducted according to OECD Test No. 473 (OECD, 1997b), EPA Health Effects Test Guidelines OPPTS 870.5375 (U.S. EPA, 1998b), and Commission Regulation (EC) No 440/2008 B10. Whilst the full study report is confidential, the study has been summarised in a publication (Clewell *et al*., 2016). Following initial cytotoxicity investigations to determine the appropriate concentrations to be used in the main tests, 2 independent experiments were conducted (each in duplicate) using V79 Chinese hamster lung (CHL) cells. In the first experiment, the CHL cells were exposed to the test article for 3 hours (short term assay; with a 20-hour sampling time) at concentrations of 250, 500, 750, and 1,000 µg/mL in the absence of S9 metabolic activation, or at concentrations of 250, 500, 750, 1,000, and 1,250 µg/mL in the presence of S9 metabolic activation. In the second experiment, the CHL cells were exposed to the test article for 20 hours (continuous assay; with a 20- or 28-hour sampling period) at concentrations of 62.5, 125, 250, and 500 µg/mL in the absence of S9 metabolic activation, or for 3 hours (with a 28-hour sampling period) at concentrations of 500, 750, 1,000, 1,250, and 1,500 µg/mL in the presence of S9 metabolic activation. Dulbecco's Modified Eagle's (DME) medium served as the negative (solvent) control. Ethyl methanesulfonate (EMS) was used as the positive control in the absence of metabolic activation, and cyclophosphamide monohydrate was used as the positive control in the

[30] Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). OJ L 142, 31.5.2008, p. 1–739.

presence of metabolic activation. At least 200 metaphase cells from each test concentration were evaluated for the presence of structural chromosomal aberrations. The number of chromatid and chromosome type aberrations as well as the number of polyploidy and endoreduplicated cells were also scored.

In both experiments, no statistically significant or dose-dependent increases in the number of cells with chromosomal aberrations were observed in cells treated with Bonolive® compared to cells treated with the negative control. In addition, no increases in the rate of polyploidy and endoreduplicated metaphases were observed. In contrast, treatment of the cells with the positive controls resulted in biologically and statistically significant increases in the number of cells with chromosomal aberrations. Thus, based on the results of this study, it was concluded that Bonolive® did not induce structural chromosomal aberrations and is non-clastogenic under the conditions of this study.

XIII.B.2.3 In vivo Mammalian Erythrocyte Micronucleus Test

The genotoxic potential of Bonolive® was further investigated in an *in vivo* mammalian erythrocyte micronucleus test conducted in rats. Whilst the full study report is confidential, the study has been summarised in a publication (Clewell *et al*., 2016). The study was conducted in accordance with OECD Test No. 474 (OECD, 1997c), Commission Regulation (EC) No 440/2008 B12, and EPA Health Effects Test Guidelines OPPTS 870.5395 (U.S. EPA, 1998c). Initially, a preliminary test was conducted to determine the doses to be tested in the main study and to assess any gender differences in response. A single dose of 2,000 mg/kg body weight Bonolive® was administered *via* oral gavage to a group of 2 male and 2 female Crl:NMRI BR mice. No mortalities, clinical signs of toxicity, or gender-specific effects were observed. Thus, the 2,000 mg/kg body weight dose was selected as the high-dose to be tested in the main study.

In the main study, groups of male Crl:NMRI BR mice were administered Bonolive® *via* oral gavage in a single dose of 0 (negative control), 500 (low-dose), 1,000 (mid-dose), or 2,000 (high-dose) mg/kg body weight. The negative control consisted of the vehicle (Humaqua). The positive control, 60 mg/kg body weight of cyclophosphamide, was administered by intraperitoneal injection. All test doses and controls were administered at a volume of 10 mL/kg body weight. The negative control and high-dose[31] groups comprised 10 animals, whereas all other groups comprised 5 animals. Bone marrow samples were prepared for 5 animals per group at 24 hours post-treatment in the low-dose, mid-dose, and positive control groups, and at 24 and 48 hours post-treatment in the high-dose and negative control groups. The frequency of micronucleated cells was scored in 2,000 polychromatic erythrocytes per animal, and the proportion of immature to total erythrocytes was determined by counting a total of at least 200 immature erythrocytes per animal.

[31] Two additional male mice were administered the high dose of 2,000 mg/kg body weight of the test article, to replace any mice that die before the scheduled sacrifice time. Since no deaths occurred in the original test population, bone marrow smears were not prepared from the additional mice as they were not used as replacements. It is worthy to note, however, that these additional animals were symptom-free during the study.

No mortalities occurred during the study. On the day of test article administration, a slight decrease in activity and piloerection was observed in 4/10 mice in the high-dose group. However, since these symptoms were not observed at 24 and 48 hours post-treatment, they were considered to be transient and not toxicologically relevant. No statistically significant differences were observed in the frequency of micronucleated polychromatic erythrocytes or proportion of polychromatic erythrocytes to normochromatic erythrocytes at all test doses compared to the negative control. Moreover, all results were reported to be within the laboratory's historical control range. In contrast, large statistically significantly increases in the frequency of micronucleated polychromatic erythrocytes were observed in the positive control group. Based on the results of this study, it was concluded that Bonolive® does not have genotoxic potential in the mouse micronucleus test at doses of up to 2,000 mg/kg body weight, the highest dose tested.

XIII.B.3 Other Studies (Non-Product Specific)

A number of repeat-dose oral toxicity, reproductive and developmental oral toxicity, and genotoxicity studies have been identified in the literature investigating the preclinical safety of various olive leaf, fruit, and pulp extracts that are not product-specific to Bonolive®. Although the composition of the investigational products tested in these studies may not be identical to Bonolive®, the results from these studies may be used to further understand and corroborate the preclinical safety of Bonolive®.

XIII.B.3.1 Repeat-Dose Studies

The details of the repeat-dose oral toxicity studies of olive leaf, fruit, and pulp extracts are summarised in Table XIII.B.3-1. In 2 studies conducted in rats, administration of the test article (olive leaf or pulp extract) at a dose of up to 1,000 or 2,000 mg/kg body weight/day for 12 to 90 days, respectively, did not result in test article-related adverse effects (Christian *et al.*, 2004; Kumral *et al.*, 2015). All observations reported in these studies were considered to be incidental or occurred at similar or higher instances in the control groups; thus, they were not considered to be toxicologically relevant.

In one rat study, some effects were observed on serum liver enzymes and lipids following the gavage administration of olive phenolic compounds at the highest concentration tested (1,600 ppm) for 7 weeks, suggesting possible liver damage (Farag *et al.*, 2003). However, since no further details were provided on the characterisation of the test article, and since the equivalent dose in mg/kg body weight/day could not be calculated, it is difficult to compare these results to those of other studies, and therefore, the relevancy is limited. Additionally, in a mouse study, administration of an olive leaf extract (D-lenolate) in the diet for 14 weeks resulted in a higher mortality rate (50%), lower body weight gain, as well as clinical chemistry, macroscopic, and histopathological findings associated with liver toxicity (*e.g.*, increase in liver weights, increase in ALT and ALP, changes in liver architecture and lesions), which were more pronounced or exclusively observed in the high-dose group (0.75% in the diet, equivalent to 1,125 mg/kg body weight/day) (Arantes-Rodrigues *et al.*, 2011). Further details on the characterisation of the test article also were not available and

therefore, it is unknown if the test article is comparable to Bonolive®. Moreover, the results from the 14-day and 90-day rat studies conducted with Bonolive® indicated that no adverse effects on the liver or evidence of liver toxicity were observed at 2,000 mg/kg body weight/day or 1,000 mg/kg body weight, respectively, the highest dose tested in each study. This suggests that the test article in the mouse study may be significantly different in composition to Bonolive®, and as such, the results from this study may not be relevant in the evaluation of the safety of Bonolive®. Furthermore, an olive leaf powder (21 g/kg dry matter oleuropein content, 67 g/kg dry matter total polyphenol content) was investigated in pigs for 6 weeks and apart from a decreased final body weight and daily weight gain observed in the high-dose group (*i.e*., 50 g/kg feed; 3,200 mg total polyphenols/kg body weight), no significant effects (*i.e.*, on organ weights, liver enzymes, and serum lipids) were observed (Paiva-Martins *et al.*, 2014).

Table XIII.B.3-1 Summary of Repeat-Dose Studies Conducted on Olive Leaf, Fruit, and Pulp Extracts (Non-Product Specific)

Species (Strain), Sex, and Number of Animals	Test Article, Route of Administration and Study Duration	Dose in mg/kg bw/d (concentration)	Parameters Evaluated	NOAEL (mg/kg bw/d)	Significant Findings[a,b]	Reference
Rats (Sprague-Dawley), M, 6/group	Olive leaf extract (Bio-Olive Ltd, 196.8 mg/g total phenols and 97 mg/g oleuropein) Oral (drinking water) 12 days	Control[c]: 0 + doxorubicin Group 1: 500 mg/kg bw/d + doxorubicin Group 2: 1,000 mg/kg bw/d + doxorubicin A single dose of 30 mg/kg doxorubicin (a drug known to increase oxidative stress in several organs) was injected IP on Day 8	Body weight, organ weights, prooxidant and antioxidant markers in the heart, liver, and kidneys, serum markers of cardiac, hepatic, and renal damage, histopathology	NR	• NSD in change in body weight • NSD in organ weights (liver, heart, kidney) • ↓ Serum cardiac troponin I, ALT, AST in all groups • NSD in urea and creatinine • Markers in the heart: ↓ Malondialdehyde, diene conjugate, and protein carbonyl levels in all groups; ↑ GSH in all groups • Markers in the liver: ↓ Malondialdehyde, diene conjugate [Group 2], and protein carbonyl levels in all groups; ↑ GSH in Group 2; ↓ SOD [Group 2] • Markers in the kidney: Liver: ↓ Malondialdehyde, diene conjugate [Group 2], and protein carbonyl levels in all groups; ↑ GSH in both groups • Reduced histopathological changes observed in both groups	Kumral *et al.* (2015)
Rats (Sprague-Dawley), M, 8/group	Phenolic compounds from fruits and leaves from the Picual cultivar olive (not further characterised) Oral (gavage) 7 weeks	Control: 0 (water/Tween) Group 1: 400 ppm Group 2: 800 ppm Group 3: 1,600 ppm BHT Group: 200 ppm of BHT Equivalent doses in mg/kg bw/d unable to be determined	Serum AST, ALT, cholesterol, total lipids	NR	• ↑ Serum AST and ALT [Group 3, BHT] • Slight ↑ in kidney and liver weights in Group 3; BHT caused significant enlargement of these organs • Histopathological changes in the kidney and liver tissues [Group 3, BHT] • ↑ Serum AST and ALT [Group 3, BHT]; NSD in other groups • ↑ HDL-CH [Groups 1, 2, and 3] from week 3, 1, and 3 onwards; ↑ HDL-cholesterol in [BHT] from week 5 onwards • ↑ Serum total lipids in [Group 3, BHT]; NSD in other groups	Farag *et al.* (2003)

Table XIII.B.3-1 Summary of Repeat-Dose Studies Conducted on Olive Leaf, Fruit, and Pulp Extracts (Non-Product Specific)						
Species (Strain), Sex, and Number of Animals	**Test Article, Route of Administration and Study Duration**	**Dose in mg/kg bw/d (concentration)**	**Parameters Evaluated**	**NOAEL (mg/kg bw/d)**	**Significant Findings[a,b]**	**Reference**
		Control: 0 (water/Tween) Group 1: 1,200 ppm Group 2: 1,600 ppm BHT Group: 200 ppm of BHT Equivalent doses in mg/kg bw/d unable to be determined	Relative organ weights, histopathology		• ↑ Relative kidney weight [BHT]; NSD in other groups • ↑ Relative liver weight [BHT]; NSD in other groups • Histopathology: Group 1 findings were similar to control rats; Group 2 findings included dilated central veins and engorged with blood, enlarged hepatocytes with granular cytoplasm, focal mononuclear leukocytic inflammatory cell aggregation between hepatocytes and adjacent to central veins, fibroblastic proliferation associated with hyperplastic activation in the epithelial cells lining the bile ducts; similar microscopic changes indicating damage to the liver and kidney tissues were observed in the BHT group	
Rats [Crl:CD (SD)IGS BR VAF/Plus], M and F, 20/sex/group	Olive pulp extract (6% polyphenol content, HIDROX™, CreAgri, Inc.) Oral (gavage) 90 days	Control: 0 (vehicle) Group 1: 1,000 mg/kg bw/d Group 2: 1,500 mg/kg bw/d Group 3: 2,000 mg/kg bw/d	Daily clinical signs, weekly body weights and feed consumption, ophthalmology, haematology and serum chemistry, macroscopic and histopathology of selected tissues	2,000	• No mortalities • NSD in body weight gain • NSD in feed consumption • ↑ WBC [M, Group 2] • ↑ RBC [F, Group 3] • ↑ CH [F, Group 3] • ↓ ALT [M, F, Groups 1, 2, 3] • ↓ SDH [F, Groups 2 and 3] • No test article-related gross or histopathological findings (all findings were attributed to local irritation by repeated intubation of large volumes of the viscous, granular dosing suspension)	Christian *et al.* (2004)

Table XIII.B.3-1 Summary of Repeat-Dose Studies Conducted on Olive Leaf, Fruit, and Pulp Extracts (Non-Product Specific)

Species (Strain), Sex, and Number of Animals	Test Article, Route of Administration and Study Duration	Dose in mg/kg bw/d (concentration)	Parameters Evaluated	NOAEL (mg/kg bw/d)	Significant Findings[a,b]	Reference
Mice (ICR), F, 10/group	Olive leaf extract (D-lenolate, East Park Research, Inc.) (not further characterised) Oral (diet) 14 weeks	Control: 0 (basal diet) Group 1: 375[d] mg/kg bw/d [0.25%] Group 2: 750[d] mg/kg bw/d [0.5%] Group 3: 1,125[d] mg/kg bw/d [0.75%]	Mortality, body weight parameters, macroscopic changes, liver enzymes (ALT, ALP, total bilirubin, albumin), absolute and relative organ weights, histopathology of liver samples	NR	• Mortality rates of 10, 0, 20, and 50% in control, Group 1, 2, and 3, respectively • NSD in food intake • NSD in the ponderal homogeneity index or ponderal gain • ↓ Final body weight [Group 3] • No observed changes in behaviour • Icterus was observed in 20% of animals in Group 2 and in 90% of animals in Group 3 • ↓ Absolute and relative spleen weights [Group 3] • ↑ Relative liver weights [Groups 2, 3] • ↓ Relative right kidney weights in all groups • ↑ ALT [Groups 2, 3] • ↑ ALP [Groups 2, 3] • No macroscopic changes in the heart, kidneys, bladder, spleen, and lungs • Macroscopic changes in the liver were observed in all test groups, and included greenish liver staining, bile duct dilatation and gall bladder distension; control livers were normal • Histopathological findings in the liver included liver architecture alterations [Groups 2, 3], hepatic fibrosis (more severe in Group 3), bile duct hyperplasia, hepatocyte necrosis and inflammatory infiltrates, and liver mitosis in all test groups; control livers did not have any findings • ↓ Respiratory indices and activities of liver mitochondrial chain complexes in Group 3	Arantes-Rodrigues *et al.* (2011)

Table XIII.B.3-1 Summary of Repeat-Dose Studies Conducted on Olive Leaf, Fruit, and Pulp Extracts (Non-Product Specific)

Species (Strain), Sex, and Number of Animals	Test Article, Route of Administration and Study Duration	Dose in mg/kg bw/d (concentration)	Parameters Evaluated	NOAEL (mg/kg bw/d)	Significant Findings[a,b]	Reference
Pigs (Large White X Landrace X Pietrain), M and F, 4/sex/group An additional 9 pigs were used to investigate digestibility	Olive leaf powder (21 g/kg dry matter oleuropein content, 67 g/kg dry matter total polyphenol content) Oral (diet; incorporated into pelleted feed) 6 weeks	Control: 0 (control diet) Group 1: 25 g/kg feed Group 2: 50 g/kg feed Total phenolic levels of 0, 1,600 and 3,200 mg/kg, respectively	Body weight, feed intake, liver function, organ weights of selected organs, total tract apparent digestibility	NR	• ↓ Final body weight and daily weight gain [Group 1] • NSD in feed intake • ↓ Energy intake [Group 1] • ↑ Feed to gain ratio in both groups • ↓ Backfat thickness in [Group 2] • NSD in carcass weight and carcass yield • NSD in dry matter, organic matter, digestibility • ↓ Crude protein and crude fat digestibility in both groups • NSD in total bilirubin, direct bilirubin, AST, ALT, ALP, GGT • NSD in TG, HDL, LDL, VLDL • NSD on organ weights (liver, heart, lungs, tongue, kidneys, perinephric adipose tissue)	Paiva-Martins *et al.* (2014)

↓ = decrease; ↑ = increase; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate transaminase; BHT = butylated hydroxytoluene; bw = body weight; CH = cholesterol; d = day; F = female animals; GGT = gamma-glutamyl transpeptidase; GSH = glutathione; HDL = high-density lipoprotein; IP = intraperitoneal; LDL = low-density lipoprotein; M = male animals; NOAEL = no-observed-adverse-effect level; NR = not reported; NSD = no significant differences; ppm = parts per million; RBC= red blood cells; SDH = sorbitol dehydrogenase; SOD = superoxide dismutase; TG = triglycerides; VLDL = very low-density lipoprotein; WBC = white blood cells.

[a] Unless stated otherwise, all reported effects are statistically significantly different relative to control group(s)

[b] Information in [ ] correspond to the dose in which the reported effects were observed.

[c] Note that an additional control group, without doxorubicin, was also presented in the study. However, this group was not tabulated since all animals in all other test groups were given doxorubicin (*e.g.*, independent effects would not able to be isolated).

[d] dose calculated using conversion table (U.S. FDA, 1993).

XIII.B.3.2 Reproductive and Developmental Toxicity

The effects of an olive pulp extract (6% polyphenol content) on reproductive and developmental parameters have been investigated in rats (Christian *et al*., 2004). The details and results from this study are tabulated in Table XIII.B.3.2-1. No test-article related adverse effects were observed on all parental/maternal, reproductive, and foetal parameters at up to 2,000 mg/kg body weight/day, the highest dose tested.

Table XIII.B.3.2-1 Summary of Reproductive and Developmental Studies on Olive Leaf Extracts (Non-Product Specific)

Species (Strain) and Number of Animals	Test Article, Route of Administration and Exposure Period	Dose in mg/kg bw/d (concentration)	Reported Effects [a,b]		Reference
Rats [Crl:CD (SD)IGS BR VAF/Plus], M and virgin F, 8/sex/group (parental), 2/sex/litter (foetal) Additional 24 rats (6/sex/group) used for TK analysis	Olive pulp extract (6% polyphenol content, HIDROX™, CreAgri, Inc.) Oral (gavage) Parental: 14 days before cohabitation to necropsy (M were euthanised after 49 days, F after the completion of the 22-day post-partum period) Foetal: Weaned 21 days post-partum, 1 week administration period, euthanised on PPD 21 or 28	Control: 0 (vehicle) Group 1: 1,000 mg/kg bw/d Group 2: 1,500 mg/kg bw/d Group 3: 2,000 mg/kg bw/d	Parental Parameters	• 1 F [1,000] died on the last day of lactation; attributed to a torsion of the right uterine horn and unrelated to the test article • Excessive salivation was observed in M and F, deemed to be a finding associated with the relative viscosity of the test article suspension • NSD in feed intake • NSD in terminal body weights, paired testes, paired epididymides • NSD in number of F pregnant, body weight gain in gestation, gestation index	Christian *et al.* (2004)
			Foetal Parameters	• NSD in mean pups delivered per litter, viability index, pup weight per litter	
			NOAEL	Parental: 2,000 mg/kg bw/d[c] Foetal: 2,000 mg/kg bw/d[c]	
Rats [Crl:CD (SD)IGS BR VAF/Plus], virgin F, 25/group Additional 18 F (6/group) used for toxicokinetic analyses	Oral (gavage) Days 6 to 20 of gestation	Control: 0 (vehicle) Group 1: 1,000 mg/kg bw/d Group 2: 1,500 mg/kg bw/d Group 3: 2,000 mg/kg bw/d	Maternal and Reproductive Parameters	• 1 premature delivery [2,000]; no abnormal findings were noted for this dam or the litter and all other rats survived until the scheduled necropsy • No adverse clinical or necropsy observations • NSD in maternal body weights, body weight gains, gravid uterine weights, corrected maternal body weights or body weight gains, absolute or relative feed consumption • ↑ Mean number of corpora lutea [2,000] (but within historical control range) • NSD in mean number of implantations, resorptions	
			Foetal Parameters:	• NSD in litter size, foetal body weights, number of foetuses with alterations/abnormalities, and litters with “altered” foetuses	

Table XIII.B.3.2-1 Summary of Reproductive and Developmental Studies on Olive Leaf Extracts (Non-Product Specific)					
Species (Strain) and Number of Animals	**Test Article, Route of Administration and Exposure Period**	**Dose in mg/kg bw/d (concentration)**	**Reported Effects [a,b]**		**Reference**
			NOAEL	Maternal: 2,000 mg/kg bw/d[c] Developmental: 2,000 mg/kg bw/d[c]	

bw = body weight; d = day; F = female animals; M = male animals; NOAEL = no-observed-adverse-effect level; NSD = no significant differences.
[a] unless stated otherwise, all reported effects are statistically significantly different relative to control group(s)
[b] numbers in [ ] correspond to the dose(s) at which the reported effects were observed
[C] determined by applicant

XIII.B.3.3 Genotoxicity

The details of the non-product specific studies evaluating genotoxicity are summarised below in Table XIII.B.3.3-1. Across the *in vitro* genotoxicity studies identified, positive results were obtained in a bacterial reverse mutation test and a chromosomal aberration test conducted with olive pulp extract at test concentrations ≥1,000 µg/plate or µg/mL (Christian *et al.*, 2004). However, it was noted by the study investigators that the interpretation of these study observations were complicated by the inconsistencies in the study results between the regular and repeat tests (*i.e.*, positive results were obtained only in the presence of metabolic activation in the confirmatory pre-incubation test), the antibacterial properties of olive pulp extract, and the observation of positive results only at 1 or 2 concentrations where precipitates also were present.

Moreover, in all of the *in vivo* genotoxicity tests identified, uniformly negative results were obtained, indicating that the test articles did not have genotoxic potential *in vivo*.

Table XIII.B.3.3-1 Summary of Other Genotoxicity Studies Conducted with Olive Leaf Extracts (Non-Product Specific)

Test	Test Article	Test System/ Animal Species	Concentration/ Dose	Results	Reference
***In Vitro* Studies**					
Bacterial reverse mutation test	Olive pulp extract (6% polyphenol content, HIDROX™, CreAgri, Inc.)	*Salmonella typhimurium* TA97a, TA98, TA100, TA1535, and *Escherichia coli* WP2 uvrA (±S9)	5 to 5,000 µg/plate (initial plate incorporation test)	Positive [at 1,000, 2,500 µg/plate in TA98 and TA100, unknown if +S9 or –S9]	Christian *et al.* (2004)
			50 to 2,500 µg/plate (confirmatory pre-incubation test)	Positive [at 1,000, 2,500 µg/plate in TA98 and TA100, +S9][a]	
Chromosomal aberration test		Chinese hamster ovary cells (±S9)	10 to 1,000 µg/mL (3 hour incubation)	Positive [at 1,000 µg/plate, +S9]	
***In Vivo* Studies**					
Micronucleus test	Olive pulp extract (6% polyphenol content, HIDROX™, CreAgri, Inc.)	Crl:CD (SD)IGS BR VAF/Plus rats, M and F, 5 to 14/sex/group	Single dose: 0, 1,000, 1,500, and 2,000 mg/kg bw/d Positive control: 50 mg/kg bw/d CP	Negative	Christian *et al.* (2004)

Table XIII.B.3.3-1 Summary of Other Genotoxicity Studies Conducted with Olive Leaf Extracts (Non-Product Specific)

Test	Test Article	Test System/ Animal Species	Concentration/ Dose	Results	Reference
			Repeat-dose: 0, 1,000, 1,500, 2,000 (all 28 days), 5,000 (29 days) mg/kg bw/d Positive control: 50 mg/kg bw/d CP	Negative[b]	
Micronucleus test	Olive leaf extract (Furfural Español S.A.)	Adult male Swiss mice, 5 to 7/group	50 g/100 mL drinking water (provided 5 days prior to X-ray irradiation)	Negative[c]	Benavente-García *et al.* (2002)
Drosophila wing spot test (somatic mutation and recombination test)	Olive leaf methanol extract	*Drosophila melanogaster* larvae (mwh/flr3)	0.8 to 12 mg polyphenols/4 mL medium	Negative	Kounatidis *et al.* (2009)
	Pure oleuropein		0.8 to 8 mg/4 mL medium		

+ S9 = with metabolic activation; - S9 = without metabolic activation; bw = body weight; CP = cyclophosphamide; d = day; F = female animals; M = male animals.
[a] Microscopic precipitate was observed at 1,000 µg/plate in TA100 (-S9). Non-interfering precipitate was observed at 1,000 and 2,500 µg/plate in TA98 (+S9), and 2,500 µg/plate in TA100 (-S9).
[b] Only the 5,000 mg/kg bw/d dose was scored.
[c] Results were negative prior to X-ray irradiation (statistical significance not provided; however, stated that value comparable to control). Post-X-ray irradiation, the test article was stated to be radioprotective and anti-clastogenic.

XIII.C Human Studies

XIII.C.1 Safety Studies Conducted with Bonolive®

A human clinical study was conducted to evaluate the bioavailability of Bonolive® and its effects on plasma antioxidant status in pre- and post-menopausal women. Whilst the full study report is confidential, the study has been summarised in a publication (García-Villalba *et al.*, 2014). Sixteen women (8 pre-menopausal, 8 post-menopausal), aged 18 to 75 years, were given a single 250 mg dose of Bonolive®. Blood and urine samples were collected for 24 hours post-administration (at 0, 1, 2, 3, 4, 6, 8, 12, 16, and 24 hours) for pharmacokinetic analyses. The results on bioavailability have previously been discussed in Section XIII.A. Although the primary objective of this study was not to evaluate safety, data on adverse events were collected throughout the study, which can be used to support the safety of Bonolive®. It was reported that no adverse events were experienced by the study subjects (*i.e.*, intolerance, nausea, dyspepsia, constipation, diarrhoea, allergic reactions, *etc*.). In addition, no adverse effects on plasma antioxidant status were observed.

Additional safety data for Bonolive® was available in a randomised, double-blind, parallel-group clinical trial in which 64 osteopenic subjects between 49 and 68 years old were randomised to receive either 250 mg/day of Bonolive® or placebo for 12 months. Whilst the full study report is confidential, the study has been summarised in a publication (Filip *et al.*,

2015). Both test groups also received 1,000 mg/day of calcium carbonate, and were instructed not to take any other calcium and vitamin supplements or parapharmaceuticals during the study period. The primary outcome measure was change in serum levels of the bone metabolism biomarkers osteocalcin and C-terminal cross-linking telopeptide of type I collagen, which are markers of bone formation and resorption, respectively. Although the primary objective of this study was not to evaluate safety, all incidences of adverse events, including information on their severity and implications, were recorded throughout the study at each clinic visit[32]. A basic physical examination, vital signs, haematology, biochemistry, and basis urine analysis were also conducted. The overall incidences of adverse events were similar between the Bonolive® and placebo groups and of the adverse events that did occur in this study, none were not considered to be clinically-relevant or treatment-related. Moreover, no adverse effects on blood lipids were observed, and no significant differences were observed between groups in the haematology parameters, aside from an increase in phosphate in the Bonolive® group, which was not considered an adverse effect. No significant or toxicologically-relevant effects were observed in the inflammatory markers CRP and IL-6 between groups, and plasma levels of 25(OH)D and serum calcium did not significantly change in any group.

Thus, based on the results from these studies, the ingestion of Bonolive® is well tolerated and not associated with any test article-related adverse events in humans.

XIII.C.2 Other Studies (Non-Product Specific)

A number of other studies have been identified in which the effects of various olive leaf extracts on parameters related to safety were investigated in human subjects. Although the composition of the olive leaf extracts tested in these studies may not be identical to Bonolive®, the results from these studies can be used to further understand and corroborate the clinical safety of olive leaf extracts. A tabulated summary of the details and results from these studies is provided in Table XIII.C.2-1. In all studies, no test article-related adverse events or serious adverse events were reported and any adverse events that were reported occurred with equal or lower frequency compared to the respective control group for each study. In addition, in studies where other parameters related to safety were measured (*e.g.*, serum lipids, inflammatory markers, liver function, liver enzymes), no significant differences were reported between groups. Thus, the results from these studies corroborate that olive leaf extracts, such as Bonolive®, are safe and tolerable in human subjects.

[32] A total of 6 clinic visits were scheduled, consisting of a first screening visit, an introductory visit (V1), a visit at start of treatment (V2) and follow-up visits at 3, 6, and 12 months of treatment (V3 to V5)

Table XIII.C.2-1 Summary of Other Human Studies Conducted on Olive Leaf Extracts (Non-Product Specific)				
Study Population	**Study Design & Intervention Duration**	**Intervention(s)**	**Results[a]**	**Reference**
46 Overweight males, 35 to 55 y, BMI 28.0±2.0 kg/m^2 43 subjects completed the study	R, DB, C, X 12 weeks per intervention (with 6 week washout)	4 capsules/day of olive leaf extract suspended in safflower oil (51.1 mg oleuropein and 9.7 mg hydroxytyrosol) 4 capsules/day of placebo (safflower oil only)	<u>Adverse events:</u> • No dropouts or adverse events were reported in the test group • NSD in subjective assessment of wellbeing NSD between groups in the liver function tests (AST, ALP, ALT, and GGT) <u>Other Relevant Parameters:</u> • ↑ Insulin sensitivity and disposition index • ↑ IGFBP-1 and 2 plasma hormones; NSD in IGF-I, IGF-II, or IGFBP-3 • ↑ IL-6 • NSD in IL-8, CRP, TNFa • NSD in plasma lipids • NSD in adiposity • NSD in ambulatory (24 hour) blood pressure • Glucose homeostasis	de Bock *et al.* (2013a)
79 Adults with type II diabetes, 51M and 28F, mean age 61± 8 y, BMI <40 kg/m^2	R, C, DB, P 14 weeks	Tablet containing 500 mg/day of a hexane and ethanolic extract of olive leaves Placebo tablet	<u>Adverse events:</u> • No adverse events reported <u>Other Relevant Parameters:</u> • ↓ HbA1c levels • ↓ Fasting insulin levels • NSD in postprandial insulin levels	Wainstein *et al.* (2012)
40 Borderline hypertensive monozygotic twins, 18 to 60 y	Open, R, C, P 8 weeks	<u>Experiment 1</u>: 500 mg/day tablet of olive leaf ethanolic extract (EFLA®943, 18 to 26% oleuropein and 30 to 40% polyphenols) Control (receiving lifestyle advice on how to reduce hypertension)	<u>Adverse events:</u> • No adverse events observed throughout the study	Perrinjaquet-Moccetti *et al.* (2008)

Table XIII.C.2-1 Summary of Other Human Studies Conducted on Olive Leaf Extracts (Non-Product Specific)				
Study Population	**Study Design & Intervention Duration**	**Intervention(s)**	**Results[a]**	**Reference**
		Experiment 2: 500 mg/day: 1 tablet of olive leaf ethanolic extract (EFLA®943) 1,000 mg/day: 2 tablets of olive leaf ethanolic extract (EFLA®943)		
232 Subjects with stage-1 hypertension, mean age ~50 y (162 subjects completed the study, 148 analysed)	R, C, DB, P 8 weeks (4-week run in period with diet alone)	500 mg/day olive leaf ethanolic extract (EFLA®943) Captopril (Dexacap®, "control drug")	Adverse events: • Incidence of adverse events was similar between groups (83 *vs*. 85 events in EFLA *vs.* control group, respectively) Other Relevant Parameters: • Similar blood pressure lowering effect between groups • Beneficial ↓ Total CH and TG levels, ↓ of LDL-CH from baseline • Slight changes in ALT, haematocrit, platelet and potassium levels in the olive group and in ALT, serum creatinine, haemoglobin, haematocrit, RBC and WBC count in the Captopril group. Not considered to be clinically relevant since absolute values for each parameter within their respective normal range and small in magnitude • NSD in other laboratory parameters	Susalit *et al.* (2011)
39 Healthy subjects, 45.0 ± 8.8 y, total CH >220 mg/dL	R, C, DB, P 28 days	1,200 mg/day of olive leaf extract (Olivia®) capsule, twice daily) Placebo capsule (twice daily)	Adverse events: • No adverse events reported Other Parameters: • NSD in plasma TG, AST, ALT, creatinine and uric acid levels • ↓ CH, LDL-CH, total CH:HDL-CH, oxidised LDL, GGT • All plasma parameters returned to normal after stopping the interventions	Fonollá *et al.* (2010) – abstract only

Table XIII.C.2-1 Summary of Other Human Studies Conducted on Olive Leaf Extracts (Non-Product Specific)				
Study Population	**Study Design & Intervention Duration**	**Intervention(s)**	**Results[a]**	**Reference**
10 Subjects, 5M and 5F, 42.8 ± 7.4 y, BMI 26.9 ± 1.9 kg/m^2 (9 subjects completed the study)	R, uncontrolled, X (within each capsule or liquid formulation) Single administration	Low-dose capsule of olive leaf extract (51.1 mg oleuropein, 9.7 mg hydroxytyrosol) High-dose capsule of olive leaf extract (76.6 mg oleuropein, 14.5 mg hydroxytyrosol) Low-dose liquid preparation of olive leaf extract (51.1 mg oleuropein, 5.4 mg hydroxytyrosol) High-dose liquid preparation of olive leaf extract (76.6 mg oleuropein, 8.1 mg hydroxytyrosol)	<u>Adverse events:</u> • No adverse events were observed <u>Other Parameters:</u> • NSD in liver function markers (AST, ALT, ALP, GGT, international normalised ratio)	de Bock *et al.* (2013b)

ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate transaminase; BMI = body mass index; C = controlled; CH = cholesterol; CRP = C-reactive protein; DB = double-blind; DNA = deoxyribonucleic acid; F = female; GGT = *gamma*-glutamyl transpeptidase; HbA1c = glycated haemoglobin; HDL = high-density lipoprotein; IGF = insulin-like growth factor; IGFBP = insulin-like growth factor binding protein; IL = interleukin; LDL = low-density lipoprotein; M = male; NSD = no significant differences; P = parallel; R = randomised; RBC = red blood cell; TG = triglycerides; TNFa = tumour necrosis factor alpha; WBC = white blood cell; X = crossover; y = years.
[a] unless stated otherwise, all reported effects are statistically significantly different relative to control group(s)

XIII.D Allergenicity

As indicated in Section X.B, allergic reactions to pollen from olive trees have been reported frequently in the literature, occurring mainly in Mediterranean areas, where *Olea europaea* L. trees are commonly found. Sensitive individuals may suffer symptoms of allergic rhinitis, conjunctivitis and asthma as a result of exposure (Moreno *et al.*, 2015). It is important to note that, as described in Section II.A.1, the leaves used to manufacture Bonolive® are either obtained in February, prior to blossoming in May, or in August, well after blossoming. As such, exposure to pollen is limited. Furthermore, the manufacturing process for Bonolive® includes several purification steps that are expected to remove potential allergens.

Contact (topical) allergy to olive oil is rare, and may result in eczema-type symptoms in sensitive individuals, although ingestion of the oil may often still be tolerated (Isaksson and Bruze, 1999; Williams and Tate, 2006). In addition, despite its common consumption, food allergy to olive fruits is extremely rare and some evidence exists to suggest that olive leaf polyphenols may be protective against certain allergic-types of reactions (*e.g.*, by inhibiting mast cell degranulation) (Persia *et al.*, 2014).

As stated in Section X.B, Bonolive® does not contain any of the allergens listed in Commission Directive 2007/68/EC[33]. Moreover, as indicated in Section I.A.4, the protein content of Bonolive® is low (*i.e.,* 1.16%). As such, no concerns regarding allergenicity are anticipated for the human consumption of Bonolive®.

[33] Commission Directive 2007/68/EC of 27 November 2007 amending Annex IIIa to Directive 2000/13/EC of the European Parliament and of the Council as regards certain food ingredients. OJ L 310, 28.11.2007, p. 11–14

OVERALL CONCLUSIONS

BioActor intends to market Bonolive® as a novel food ingredient in functional foods, food supplements and foods for special medical purposes in the EU. Bonolive® is an olive leaf extract standardised to contain a minimum of 40% oleuropein and is derived from the leaf of *Olea europaea* L. Appropriate product specifications for identity and potential contaminants have been established for this ingredient, and the results of batch analyses indicate that the manufacturing process produces a consistent product free from heavy metal, microbial, and pesticide contamination.

Bonolive® is proposed for use as an ingredient in a range of food categories, including yoghurts, confectionery, fine bakery wares and beverages. In addition, it is intended for use in food supplements and foods for special medical purposes (FSMPs). As FSMPs are considered case by case for use in finished products, the safety and suitability within the context of the whole food would be justified in a notification to a member state wherein the appropriate marketing requirements would be determined. Products containing this ingredient would be targeted towards individuals over 50 years of age.

The safety of Bonolive® is supported by product specific preclinical and human studies, as well as non-product specific studies on various olive leaf extracts and powders. Metabolic fate data demonstrate that the phenolic compounds are highly bioavailable and the absorption, metabolism, and renal clearance of phenolic compounds from olive leaves is relatively rapid. Following a 14-day range-finding oral toxicity study, a 90-day oral toxicity study was conducted in rats using Bonolive® wherein no adverse effects were observed at doses of 360, 600, and 1,000 mg/kg body. Based on the results of this study, the NOAEL for Bonolive® was determined to be 1,000 mg/kg body weight/day, the highest dose tested. The results of a bacterial reverse mutation test, an *in vitro* chromosomal aberration test, and an *in vivo* mammalian erythrocyte micronucleus test demonstrate that Bonolive® is not genotoxic. The non-product specific studies corroborate the safety of Bonolive®. Thus, given that the 95th percentile intakes of Bonolive® from its cumulative exposure from foods and beverages, as well as food supplements containing Bonolive®, for the target population in adults (based on the refined assessment using the UK NDNS) is 5.3 mg/kg body weight/day, there exists a 188-fold safety factor compared to the NOAEL of 1,000 mg/kg body weight/day, the highest dose tested. In the non-target population, a worst-case 95th percentile intakes of 9.3 mg/kg body weight/day (in children) was estimated, assuming that 100% of foods and beverages in which Bonolive® is proposed for use contained the ingredient at the maximum use level and that all these foods were consumed by the non-target population. Even in this worst-case hypothetical scenario, the margin of safety remains sufficient, at 108.

Furthermore the worst case exposure of olive polyphenols from Bonolive® is well within that consumed from olives and olive oil per day as part of the “Mediterranean diet”.

These intake levels are further supported by clinical studies using Bonolive® which demonstrated that this ingredient is well tolerated and without serious adverse effects in

humans at doses of at least 250 mg/day. Additional non-product specific clinical studies corroborate the tolerability of Bonolive® as an olive leaf extracts.

Collectively, the scientific evidence presented herein demonstrates that BioActor's Bonolive® would not produce adverse health effects on human health under the intended conditions of use in conventional foods and food supplements.

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