Notice to US Food and Drug Administration of the Conclusion that the Intended Use of Bonolive® is Generally Recognized as Safe

Submitted by the Notifier:

BioActor B.V.

June 24, 2022

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Part 1: Signed Statements and Certification

1.1 Submission of GRAS Notice

BioActor B.V. is submitting a new GRAS notice in accordance with 21 CFR part 170, subpart E, regarding the conclusion that Bonolive®, a proprietary dry extract of olive (*Olea Europaea* L.) leaves, containing at least 50% polyphenols and 40% oleuropein is Generally Recognized as Safe (GRAS) for its intended use, consistent with section 201 (s) of the Federal Food, Drug and Cosmetic Act.

1.2 Name and Address of the Notifier

BioActor B.V.
Gaetano Martinolaan 50
6229 GS Maastricht
Netherlands

1.3 Name of the Substance

Dry extract of Olea europaea L. leaves containing at least 40% Oleuropein.

1.4 Intended Conditions of Use

Bonolive® is intended to be used as an ingredient in the food categories listed in Table 1 at the addition levels specified per food category. Bonolive® is not intended for use in infant formula, meat, poultry, egg products, catfish, or any products that would require additional regulatory review by USDA.

Table 1. Bonolive® Intended Uses*

Food Category	Maximum Use (ppm)
Yogurts	1111 ppm
Flavored Milk Drinks	1042 ppm
Dry Powdered Milk and Milk Mixtures (Not Reconstituted)	8333 ppm
Coconut Beverages	1042 ppm
Cookies (Certain Categories)	8333 ppm
Cereal, Granola and Nutrition Bars	8333 ppm
Fruit Juices and Nectars (Including Citrus)	1042 ppm
Vegetables and Vegetable Juices (e.g., Carrot and Tomato Juice)	1042 ppm
Fruit-Flavored Beverages (Ready to Drink and from Powders)	1042 ppm
Vegetable and Fruit Juice Blends	1042 ppm
Fortified Water	1042 ppm
Teas and coffees	1042 ppm

Nutrition Drinks and Powders	1042 ppm
Sports Drinks	1042 ppm
Table Fats and Vegetable Oils	16667 ppm
Candies (Dark Chocolate, Gum Drops, Hard Candy, Dietetic Candy)	8333 ppm
Chewing Gum	83333 ppm

*See Appendix A for a full list of food categories

1.5 Statutory Basis for GRAS Conclusion

The conclusion of GRAS status of Bonolive® for its intended conditions of use, stated in Part 1.4 of this notice, has been made based on scientific procedures.

1.6 Not Subject to Premarket Approval

We have concluded that Bonolive® is GRAS for its intended conditions of use, stated in Part 1.4 of this notice, and, therefore, such use of Bonolive® is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act.

1.7 Data and Information Availability Statement

The data and the information that serve as the basis for this GRAS conclusion will be available for review and copying during customary business hours at the office of BioActor at the address below or will be sent to FDA upon request.

BioActor B.V.
Gaetano Martinolaan 50
6229 GS Maastricht
Netherlands

1.8 Exemption from Disclosure under the Freedom of Information Act

None of the information in Parts 2 through 7 of this GRAS notice is considered exempt from disclosure under the Freedom of Information Act (FOIA) as a trade secret, personal privacy information or financial information that is privileged or confidential.

Personal privacy information is present in Part 1 of this GRAS notice.

1.9 Certification of Completion

We hereby certify that, to the best of our knowledge, this GRAS notice is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to us and pertinent to the evaluation of the safety and GRAS status of the use of Bonolive®.

_____

Antonie J. van der Saag
Managing Director
BioActor B.V.

Date: 24 June 2022

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We hereby certify that, to the best of our knowledge, this GRAS notice is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to us and pertinent to the evaluation of the safety and GRAS status of the use of Bonolive®.

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Antonie J. van der Saag Date: 24 June 2022

Managing Director

BioActor B.V.

Part 2: Identity, Manufacture, Specifications, and Physical or Technical Effect

2.1 Identification

Olea europaea L. is a dicot tree that is cultivated in a number of areas throughout the world. It is an especially important fruit tree in the European and African countries bordering the Mediterranean Sea, where it is commercially grown on more than 23 million acres.[1,2] It can also be found growing in the United States (California), as well as in Chile, Argentina, South Africa, and, Australia.[1] There is written evidence that the tree has coexisted with humans for up to 6000 years, and olive fruit pits and wood fragments have been found in ancient tombs along the eastern Mediterranean Coast.[1] The trees have oblong leaves that are up to approximately four inches long and one inch wide. The trees can live for hundreds of years, and the main products extracted from them include olives and subsequently olive oil.[1] Olive oil is one of the major components of the Mediterranean diet, which has been widely studied with regard to its benefits to human health.[3,4] According to the United States Department of Agriculture's Plant Database, *Olea europaea* L. is classified as follows:

Kingdom: Plantae
Subkingdom: Tracheobionta
Superdivision: Spermatophyta
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Asteridae
Order: Scrophulariales
Family: Oleaceae
Genus: *Olea* L.
Species: *Olea europaea* L.
Variety: *Olea europaea hojiblanca*
Olea europaea picual
Olea europaea aberquina

The tree is rich in phenolic compounds, and there has been growing interest in these constituents due to their antioxidant and subsequent potential for health benefits.[2,5] Phenolic compounds that have been identified in the *Olea europaea* L. trees include: oleuropein (a secoiridoid, see figure 1), secoiridoid derivatives (e.g. elenolic acid), 3,4-DHPEA-EDA (the dialdehydic form of elenolic acid linked to hydroxytyrosol), 3,4-DHPEA-EA (oleuropein aglycone) and p-HPEA-EDA (the dialdehydic form of elenolic acid linked to tyrosol), verbascoside, flavones (e.g. luteolin-7-*O*-glucoside, apigenin-7-*O*-glucoside, diosmetin-7-glucoside, luteolin and diosmetin), flavonols

(rutin), flavan-3-ols (catechin), phenyl and phenolic acids (e.g. tyrosol, hydroxytyrosol-see figure 2, vanillin, vanillic acid, *p*-coumaric acid and caffeic acid), and lignans (e.g. pinoresinol and acetoxypinoresinol).

Oleuropein is the most abundant phenolic compound in olive leaves, followed by the closely related hydroxytyrosol; and luteolin-7-glucosides, apigenin-7-glucosides and verbascoside have also been identified.[5-7] Cultivar, geographic region, age of the tree, and agricultural and processing techniques are important factors with regard to phenolic composition and concentration.[2,8]

Olive oil is well known for its polyphenol content. Interestingly, the leaves contain much higher concentrations of some polyphenols compared to olive oil. For example the oleuropein concentration in olive oil ranges from 0.005–0.12%, but is as high as 1–14% in olive leaves.[5,9] This is in part because the maturation and processing/fermenting of olives and olive oil causes oleuropein to hydrolyze to tyrosol and hydroxytyrosol.[8,10,11] Oleuropein can also decompose into hydroxytyrosol and elenolic acid by different factors such as light, acid, base, oxidants and high temperatures.[12]

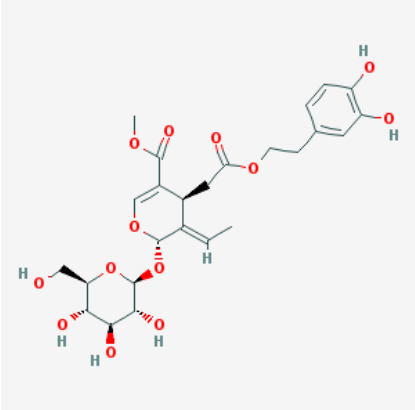


Figure 1. Molecular Structure of Oleuropein[13]

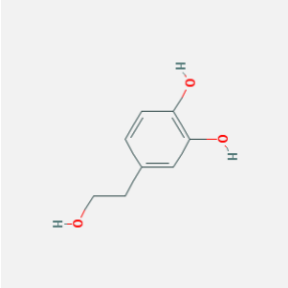


Figure 2. Molecular Structure of Hydroxytyrosol[14]

The biological activities exerted by olive phenolics in general, including those specifically from olive leaves, have demonstrated a number of potential health-promoting effects both in vivo and in vitro.[8,11,15-19] Possible mechanisms of action related to these effects may include free radical scavenging, as well as down-regulation/interaction with proatherogenic, cancer-related, and insulin-sensitivity genes.[11,20-26]

Garcia et al. investigated the chemical composition of olive leaves with regard to their use as feed for goats and sheep as lignocellulosic material.[27] They found the leaves to be rich in cell walls, gross energy, and phenolic compounds, and low in crude protein. The essential amino acid values were similar in the leaves compared to a dried olive cake that was used as a comparison. The highest values of individual amino acids were for leucine, valine, threonine (or arginine; there is a discrepancy in the paper) and alanine. Limiting amino acids could be methionine, cysteine, and tyrosine. The authors concluded that olive leaves, when used with adequate supplementation, could be of great importance as animal feed in semi-arid Mediterranean countries that have a shortage of natural pastures.

2.2 Manufacturing

2.2.1 Manufacturing Overview

Bonolive® is a proprietary extract of olive (*Olea Europaea* L.) leaves, standardized to at least 40% oleuropein, as determined by normal phase liquid chromatography using European Pharmacopoeia methods. While oleuropein is the primary polyphenol in the extract, several other polyphenols have been identified in low amounts, as is discussed below. The remainder of the ingredients consists of other major and minor components of olive leaves, such as carbohydrates, proteins, and minerals.

Olive leaves are cut and extracted using multiple extraction and purification steps as is shown in the flow chart below. The concentrated extract is spray dried to obtain the final product as a powder.

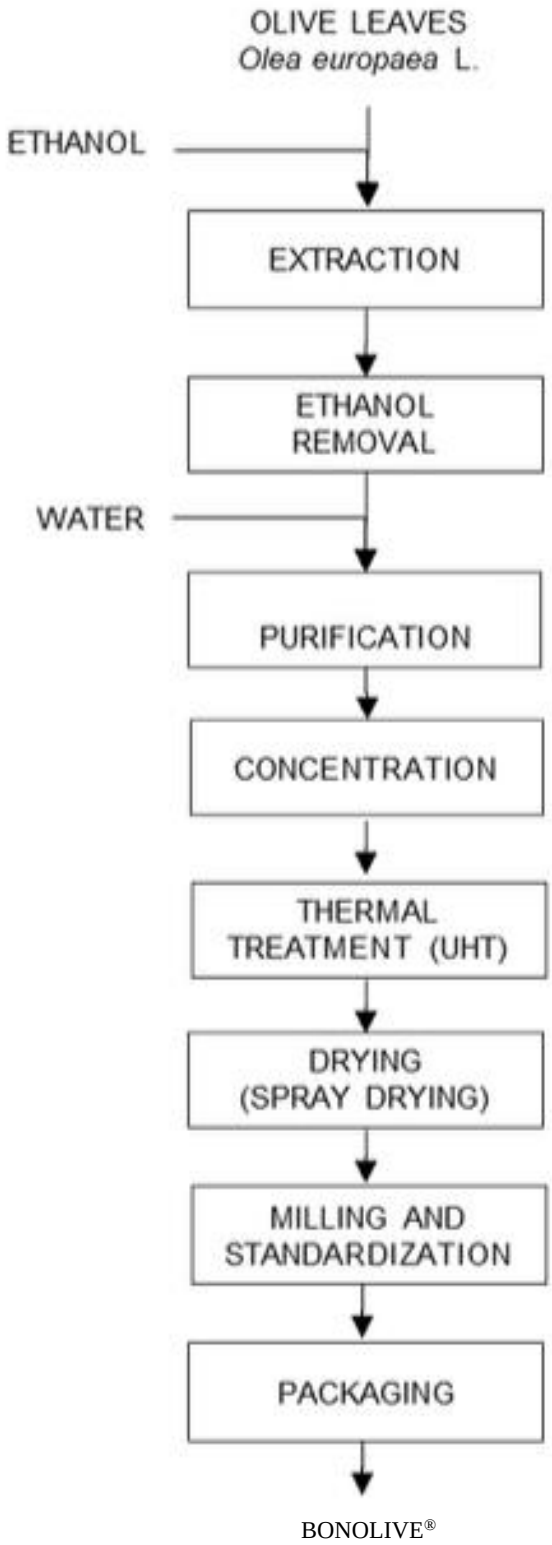


Figure 3. Manufacturing Flowchart

2.2.2 Good Manufacturing Practice

Bonolive® is manufactured in accordance with food grade and food safety standards as embraced by the Global Food Safety Initiative (FSSC 22000). Bonolive® is produced according to an established and validated HACCP plan.

2.2.3 Raw Materials

The *Olea europaea* L. leaves used in the production of Bonolive® are obtained from trees cultivated exclusively in Spain, specifically Andalusia. The *Olea europaea* L. varieties used for the production of Bonolive® are *"Olea europaea hojiblanca", "Olea europaea picual"* and *"Olea europaea aberquina".*

The olive trees from which the leaves are taken are farmed mainly for olive and olive oil production. The trees are pruned twice per year in February and August, and the pruned leaves obtained in February are generally used to manufacture Bonolive®.

2.3 Specifications

The specifications of Bonolive® along with the analytical methods are listed in Table 2 below.

Table 2. Bonolive® Specifications

Test Items	Specification	Method
Appearance	Green to brown powder	Internal
Botanical part used	*Olea Europaea* L. (leaf)	N/A
Loss on drying	8% max	Eu. Pharm c.v. (2.8.17)
Residue by calcination	9% max	Eu. Pharm c.v. (2.4.16)
Total polyphenols	50% min	French Pharmacopoeia X edition. Monografic "Vigne rouge (sec)"
Oleuropein	40% min	European Pharmacopoeia (Eu. Pharm) 04/2009:2313 (HPLC method)
Residual ethanol	1000 ppm max	Eu. Pharm c.v. (2.4.24)
Heavy Metals		
Lead	3 ppm max	Eu. Pharm. V.v. (2.4.27)
Cadmium	1 ppm max	Eu. Pharm. V.v. (2.4.27)
Mercury	0.1 ppm max	Eu. Pharm. V.v. (2.4.27)
Arsenic	2 ppm max	Eu. Pharm. V.v. (2.4.27)
PAHS		
Benzo(a)pyrene	10.0 ppb max	GC-MS
Sum of benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene and chrysene	50.0 ppb max	GC-MS

Microbiological Tests		
Total plate count	10^3 cfu/g max	Eu. Pharm. V.v. (2.6.12)
Yeast & mold	10^2 cfu/g max	Eu. Pharm. V.v. (2.6.12)
Enterobacteriaceae	10^2 cfu/g max	Eu. Pharm. V.v. (2.6.31)
Escherichia coli	Absence/1 g	Eu. Pharm. V.v. (2.6.31)
Salmonella	Absence/ 25 g	Eu. Pharm. V.v. (2.6.31)
Staphylococcus aureus	Absence/g	Eu. Pharm. V.v. (2.6.31)
Coliforms	Absence/g	ISO 4832:2006

Ph.Eur.=European Pharmacopoeia; cfu, colony forming units.

2.3.2. Methods of analysis

2.3.2.1. Total polyphenols

The total polyphenol percentage of Bonolive® is calculated according to the French Pharmacopoeia. The reference substance used is Pyrogallol >98% (ALFA-AESAR), while the solvents and reagents are water (HPLC, CAS 7732-18-5), methanol (HPLC, CAS 67-56-1), sodium carbonate R (150mg/ml CAS 497-19-8) and a reagent for phenol according to Folin-Ciocalteu. The detection wavelength is 715 nm.

For the sample solution, an amount of 20 mg of polyphenols in a 100 mL volumetric flask should be obtained by weighing the appropriate dry extract and filling to the mark with water. 5 mL of that solution should be diluted to 25 mL with water. 5 mL of the last solution should be mixed with 1 mL of reagent for phenol according to Folin-Ciocalteu in a 50 mL volumetric flask. The flask should be filled to the mark with an aqueous solution of sodium carbonate R (150 g/L). Two minutes after the addition of the last reagent, the absorbance at 715 nm can be measured, using water as the compensation liquid.

For the reference solution, approximately 50 mg of pyrogallol should be weighed and diluted in a 100 mL volumetric flask with water. 5 mL of that solution should be diluted to 100 mL with water. 5 mL of the last solution is mixed with 1 mL of reagent for phenol according to Folin-Ciocalteu in a 50 mL volumetric flask. An aqueous solution of sodium carbonate R (150 g/L) should be used to fill to the mark. Two minutes after the addition of the last reagent, the absorbance can be measured at 715 nm using water as the compensation liquid.

The formula to calculate the total polyphenols is the following:

$$\% \text{ Total polyphenols (as pyrogallol)} = \frac{13.12 \; x \; A1}{A2 \; x \; m \; x \; 2.5 \; x \; R}$$

where,

A1 = Absorbance of the sample

A2 = Absorbance of the reference substance

m = weight of the sample to be analyzed (g)

R = Purity of the reference substance as a decimal fraction

2.3.2.2. Oleuropein

The oleuropein percentage of Bonolive® is assessed by using HPLC and a method derived from the European Pharmacopoeia. The reference substance used is Oleuropein, >95%, Chromadex, USA, while the solvents and reagents are water (HPLC, CAS 7732-18-5), methanol (HPLC, CAS 67-56-1), and Trifluoroacetic acid (CAS76-05-1). The detection wavelength is 233nm and the HPLC column used is C18 (length: 15cm; internal diameter: 4,6 mm; particle size 5μm).

For the sample solution, the necessary amount of sample to obtain 0.3-0.4 mg/mL oleuropein in a 100 ml volumetric flask should be weighed. Methanol should be used for dissolving and dilution to 100 ml.

For the reference solution, approximately 10 mg of the reference substance should be weighed to a 25 ml volumetric flask. Methanol should be used for dissolving and dilution to 25 ml.

The formula to calculate the oleuropein is the following:

$$\% \text{ Oleuropein} = \frac{CC\ std\ x\ A\ test\ x\ \%R}{CC\ test\ x\ A\ std}$$

where,

CC std = Concentration of oleuropein in reference solution (mg/ml)

A test = Area of the problem peak in test sample

%R = Purity of standard (%)

CC test = Concentration of the sample (mg/ml)

A std = Area of the standard peak in reference solution

2.4 Physical or Technical Effect

Bonolive® is intended to be added to the foods listed in the intended use section as a source of polyphenols.

2.5 Batch Analyses

2.5.1 Analysis of Batches

Production conformity and consistency of Bonolive® is tested in production lots. As shown in Table 3 below, batch analyses are reasonably consistent and meet all product specifications.

Table 3. Bonolive® Batch Analysis

Test Items	Specification	Batch Number				
		PF0348220321	**PF0600130720**	**PF1138260520**	**PF1043200320**	**PF1224240720**
Appearance	Green to brown powder	Complies	Complies	Complies	Complies	Complies
Botanical part used	*Olea Europaea* L. (leaf)	Complies	Complies	Complies	Complies	Complies
Loss on drying	8% max	1.72%	2.05%	1.57%	2.06%	0.64%
Residue by calcination	9% max	0.5%	1.5%	1.1%	Complies	Complies
Total polyphenols	50% min	55.9%	51.6%	53.1%	54%	55.5%
Oleuropein	40% min	42.3%	40.9%	40.3%	40.04%	41.8%
Residual ethanol	1000 ppm max	34.7 ppm	33 ppm	31.7 ppm	39.3 ppm	57.9 ppm
Heavy Metals						
Lead	3 ppm max	Complies	Complies	Complies	Complies	Complies
Cadmium	1 ppm max	Complies	Complies	Complies	Complies	Complies
Mercury	0.1 ppm max	Complies	Complies	Complies	Complies	Complies
Arsenic	2 ppm max	Complies	Complies	Complies	Complies	Complies
PAHS						
Benzo(a)pyrene	10.0 ppb max	Complies	Complies	Complies	Complies	Complies
Sum of benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene and chrysene	50.0 ppb max	Complies	Complies	Complies	Complies	Complies
Microbiological Tests						
Total plate count	10^3 cfu/g max	Complies	Complies	Complies	Complies	Complies
Yeast & mold	10^2 cfu/g max	Complies	Complies	Complies	Complies	Complies
Enterobacteriaceae	10^2 cfu/g max	Complies	Complies	Complies	Complies	Complies
Escherichia coli	Absence/1 g	Complies	Complies	Complies	Complies	Complies
Salmonella	Absence/ 25 g	Complies	Complies	Complies	Complies	Complies
Staphylococcus aureus	Absence/g	Complies	Complies	Complies	Complies	Complies
Coliforms	Absence/g	Complies	Complies	Complies	Complies	Complies

2.5.2 Residual Solvent Analysis

Residual solvent analysis is routinely performed on every batch of Bonolive® and results have always fallen well below the established limit of 1000ppm. Ethanol is a class 3 solvent according to USP 467 (and ICH) guidelines. Class 3 solvents are not considered human health hazards. It is considered that ethanol amounts of 5000 ppm are acceptable.[28,29]

2.5.3 Residual Pesticide Analysis

Because the farmers' main intent is to preserve the olive fruits under healthy growth conditions to produce as much and as high quality of oil as possible, the trees occasionally need to be treated with pesticides to avoid pests after the flowers have been transformed into small olives. Bonolive® is manufactured using the leaves from mainly the February pruning, which have never been exposed to treatment with pesticides, making it highly unlikely that pesticide testing would lead to positive results.

Regardless, in accordance with internal standard operating procedures, pesticide residue analysis is performed on every Bonolive® batch by an external, accredited laboratory. Testing for over 450 different pesticide residues is performed, covering those used for olive tree treatment and more. Residue limits established in Regulation (EC) No 396/2005 and amendments are used as specification limits for the ingredient.

2.5.4. Contaminant Analysis

Contaminant analysis is performed periodically in production batches, based on a control program established by BioActor BV. Ethylene oxide is analyzed in Bonolive® with a maximum 0.02ppm. Pyrrolizidine alkaloids are also analyzed in Bonolive® with a maximum of 400 ppb.

2.5.5 Shelf–Life Stability

The technical data sheet for Bonolive® details that the ingredient should be stored in "tight containers to prevent dust formation, in a dry and cool place, away from direct sunlight" and "away from ignition, heat, or electricity sources." The retest date is considered to be three years.

A three-year shelf–life stability test was performed on three different Bonolive® batches under general storage conditions in a warehouse (i.e., "storehouse" conditions). Five kilograms of Bonolive® were packed in double plastic bags. This simulates the system used in commercial batches. Total polyphenols, oleuropein, verbascoside, loss on drying and microbiological values were measured at baseline and then again after three years (36 months).

The measurements were stable and within specifications throughout the study with no significant changes occurring in the parameters assayed. Results from the analyses are summarized in Table 4 below.

Table 4. Stability Study data

Parameters		T=0	T=3 years
Batch PF0613131118			
Polyphenols (%)	Min 50,0%	56,1	55,1
Oleuropein (%)	Min 40,0%	41,6	40,4
Verbacoside (%)	Min 0,5%	0,68	0,6
TAMC (cfu/g)	<10000	<50	<50
TYMC (cfu/g)	<100	<50	<50
Enterobacteriaceae (cfu/g)	<100	<10	<10
Escherichia coli	Absence/g	Absent	Absent
Salmonella	Absence/25g	Absent	Absent
S. aureus (cfu/g)	<10	Absent	Absent
Loss on drying (%)	≤8,0	2,55	2,7
Batch PF0631101218			
Polyphenols (%)	Min 50,0%	57,4	56,3
Oleuropein (%)	Min 40,0%	44,66	43,5
Verbacoside (%)	Min 0,5%	0,7	0,6
TAMC (cfu/g)	<10000	<50	<50
TYMC (cfu/g)	<100	<50	<50
Enterobacteriaceae (cfu/g)	<100	<10	<10
Escherichia coli	Absence/g	Absent	Absent
Salmonella	Absence/25g	Absent	Absent
S. aureus (cfu/g)	<10	Absent	Absent
Loss on drying (%)	≤8,0	2,94	3,1
Batch PF1925250619			
Polyphenols (%)	Min 50,0%	53,9	52,5
Oleuropein (%)	Min 40,0%	43,68	42,4
Verbacoside (%)	Min 0,5%	0,69	0,6
TAMC (cfu/g)	<10000	<50	<50
TYMC (cfu/g)	<100	<50	<50
Enterobacteriaceae (cfu/g)	<100	<10	<10
Escherichia coli	Absence/g	Absent	Absent
Salmonella	Absence/25g	Absent	Absent
S. aureus (cfu/g)	<10	Absent	Absent
Loss on drying (%)	≤8,0	2,43	2,71

2.5.6 Nutritional Analysis

The typical nutritional values of Bonolive® are listed below, in table 5.

Table 5. Nutritional values of Bonolive®

Description	Result	Unit
Nutritional value (calculated)	381.3	kCal/100g of product
Total Fat Content	0.3	g/100g of product
Saturated Fatty Acids	56.4	% Of fatty acids

Total Carbohydrates	94.4	g/100g of product
Assimilable carbohydrates	93.1	g/100g of product
Total Sugar (as Glucose)	4.2	g/100g of product
Total Fibers	1.3	g/100g of product
Total Protein	0.9	g/100g of product
Sodium	62.7	mg/100g of product

2.6 Polyphenol Analysis

The polyphenol profile of Bonolive® has been analyzed in a number of batches; the results of one analysis are summarized in Table 6 below. The company notes that while this is a reasonably typical analysis, quantitative results of polyphenol analysis vary depending upon the laboratory performing it, the techniques and methods used, the variability in reference standards utilized, etc., and thus results should be considered qualitative.

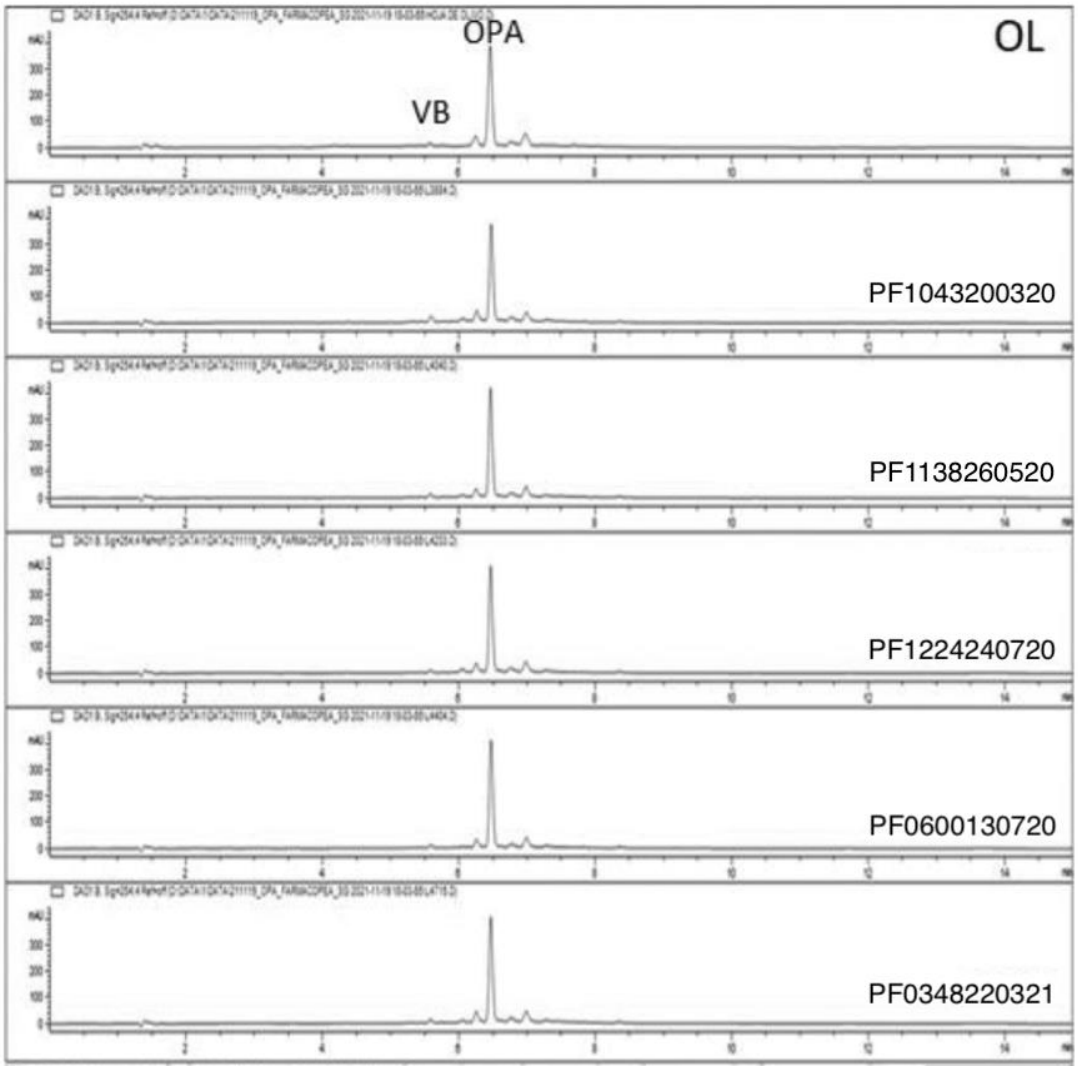
Table 6. Bonolive® Polyphenol Analysis

Polyphenol	**Relative percentage**
Verbascoside	2.34
Oleuropein	76.06
Luteolin-7-glucoside	2.03
Luteolin	0.59
Quercetin	0.03
Unquantified polyphenols	18.95
Total	100.00

2.7 Chromatographic profile

The chromatographic profile of several Bonolive® batches has been compared with the chromatographic profile of an olive leaf (figure 4). Whereas OPA; oleuropein, Vb; verbascoside and OL; olive leaf. The chromatographic profiles of Bonolive® batches presented in table 3 are almost identical with the chromatographic profile of the olive leaf, meaning that Bonolive® is substantially equivalent with a typical olive leaf.

Figure 4. Chromatographic profile of Bonolive®



2.8 Other Certifications

Bonolive® is not derived from, is not produced using, and does not come in contact with animal origin materials at any stage of its manufacturing process. There are no specific risk materials as defined in the European Commission Decision 97/534/EC and the European Pharmacopoeia Monograph 1483, "Products with risk of transmitting agents of animal spongiform encephalopathy".

Bonolive® is not genetically modified and is not derived from a genetically modified organism as defined by the EC regulations 1831/2003/EC on labeling and traceability and 1829/2003/EC on genetically modified food and feed and their amending legislation.

Bonolive® does not contain any of the allergens listed in EU Commission Directive 2007/68/EC: cereals containing gluten (with some noted exceptions), crustaceans, eggs, fish (with some noted exceptions), peanuts, soybeans (with some noted

exceptions), milk (with some noted exceptions), nuts (with some noted exceptions), celery, mustard, sesame seeds, sulphur dioxide and sulphites at concentrations of more than 10 mg/kg or mg/L expressed as SO_2, lupin and mollusks.

Bonolive® does not contain any doping substances included in WADA (World Anti-Doping Agency) prohibited list.

Bonolive® is not subjected to irradiation at any stage of the manufacturing process, as defined by EC regulations 1999/2/EC and 1999/3/EC.

Bonolive® is not manufactured using nanotechnology, does not contain nanomaterials, and/or come in contact with any nanomaterials during storage and transportation, as defined by regulations EU 1363/2013, 2283/2015, and 1169/2011.

Part 3: Dietary Exposure

Bonolive® is intended to be used as an ingredient in food where standards of identity are allowed in the categories and at the concentrations specified in Table 7 below (identical to Table 1 above). Bonolive® is not intended for use in infant formula, meat, poultry, eggs, catfish, or any products that would require additional regulatory review by the USDA.

Table 7. Bonolive® Intended Uses*

Food Category	Maximum Use (ppm)
Yogurts	1111 ppm
Flavored Milk Drinks	1042 ppm
Dry Powdered Milk and Milk Mixtures (Not Reconstituted)	8333 ppm
Coconut Beverages	1042 ppm
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Cereal, Granola and Nutrition Bars	8333 ppm
Fruit Juices and Nectars (Including Citrus)	1042 ppm
Vegetables and Vegetable Juices (e.g., Carrot and Tomato Juice)	1042 ppm
Fruit-Flavored Beverages (Ready to Drink and from Powders)	1042 ppm
Vegetable and Fruit Juice Blends	1042 ppm
Fortified Water	1042 ppm
Teas and coffees	1042 ppm
Nutrition Drinks and Powders	1042 ppm
Sports Drinks	1042 ppm
Table Fats and Vegetable Oils	16667 ppm
Candies (Dark Chocolate, Gum Drops, Hard Candy, Dietetic Candy)	8333 ppm
Chewing Gum	83333 ppm

*See Appendix A for a full list of food categories

Exposure estimates combine data on the quantity of a particular food category that is consumed with the intended concentration level of an ingredient to be added to that food category. Crème Food Safety software (www.cremeglobal.com) was used for the statistical analysis related to estimated consumption levels of Bonolive® Creme software is a probabilistic modeling tool that uses high-performance computing to predict intake (including total aggregate exposure) of food groups and/or individual ingredients. Creme Food Safety performs calculations using large scale food consumption data sets; in this case, the U.S. National Health, and Nutrition Examination Surveys' (NHANES) What We Eat in America (WWEIA) data sets, which are released every two years. NHANES uses a non-consecutive two-day, 24-hour dietary-recall protocol for data collection. In the current assessment, data from individual dietary records from Day 1 and Day 2 of NHANES survey were utilized within the Creme software.

It should also be noted that this type of daily intake methodology is generally considered to be a 'worst case' scenario as a result of several conservative

assumptions made in the consumption estimates. In addition, it is well established that the length of a dietary survey affects the estimated consumption of individual users. Short-term surveys tend to overestimate the level of the average daily intake among consumers, especially at the extremes of distribution.[30,31]

Estimates derived from Creme of the total aggregate exposures to Bonolive® were performed at both the mean and 90th percentiles. Exposure data is shown for "Food Consumers", which includes only data from individuals who reported consuming one or more of the specified food categories over the 2-day survey period. Results are given as absolute consumption (mg/day) and as consumption relevant to body weight (mg/kg bw/day). The latter estimates were based on each individual's body weight from the survey, as opposed to average body weights. Calculations also incorporated the NHANES assigned "sample weights" for each individual in the survey, which relates to the number of people in the population represented by that specific person, helping ensure that the results are representative of the entire U.S. population. Sample weights for NHANES participants incorporate adjustments for unequal selection probabilities and certain types of non-response, as well as an adjustment to independent estimates of population sizes for specific age, sex, and race/ethnicity categories.

Because data from the NHANES short 2-day survey may not adequately represent individual usual long-term intake due to the large amount of random error (e.g., intra-individual variation over time is not accounted for), estimation of "usual" or "lifetime" exposure was also added to the model, based on the methodologies developed by Nusser et al., 1996, at Iowa State University.[31] This lifetime data was considered the most relevant data, as GRAS exposure estimates should be based on expected regular exposure over the lifespan. The technique of estimating usual/lifetime intakes relies on the ability to transform the input data into normality, which is tested using the Anderson-Darling test statistic within the Creme software. If "lifetime return values" are zero or less, they may still be utilized; however, caution should be used in interpreting the data based on the nature of the warning that was received by the software. In the data shown in the tables below, all values were zero or less, and specific warnings are noted with asterisks.

The relative standard error (RSE, calculated by dividing the standard error of the estimate by the estimate itself and multiplying by 100), is a statistical criterion that can be used to determine the reliability of estimates as pertains to the population; the larger the RSE, the less reliable the estimate.[32] RSE values of greater than 25–30% are often considered a reasonable cut-off by which to consider a value unreliable.[32,33] For the purpose of this GRAS conclusion, an RSE value of greater than 25% was used to indicate that the estimated value was unreliable with regard to representing the population. RSE values are shown in the exposure estimate tables in the respective exposure sections below for the 90th percentile daily average values only, as the 90th percentile values are the most pertinent for the exposure estimates. All of the values except one in the tables were considered reasonably reliable using

the 25% cut-off. Standard errors are not calculated for lifetime exposure data, so RSE values could not be calculated for them.

Because of the large number of intended use food categories, it is nearly impossible that an individual will randomly or intentionally consume Bonolive® every time they consume one of the intended use food categories daily over a lifetime. While food labels will list Bonolive® as an ingredient and may highlight the ingredient, it is assumed that many consumers will not always realize that the ingredient is present in the food (in other words, it will likely be "invisible" in various food categories to many consumers). Additionally, Bonolive® will often be added to foods at levels that are lower than the maximum intended use levels due to formulation challenges because of the extremely bitter taste of the ingredient (as discussed in Part 4 of this document). Lastly, there will be cost and market share limitations to adding this specialty brand ingredient to foods in general, making it even less likely that an individual will consume it in all intended use food categories daily. Thus, assuming that individuals would consume the maximum addition level of Bonolive® each time they consumed any of the intended food categories listed in Table 7, will lead to a gross over-estimation of exposure.

For the above reasons, calculations were performed using both a 100% presence probability factor as well as a 20% presence probability factor for the exposure calculations. In other words, calculations were performed using the Creme software such that each of the intended use food categories was assigned a 100% or a 20% random chance of containing Bonolive® at the maximum addition level. The 20% presence probability factor was considered to result in a more reasonable and yet still highly conservative estimation of exposure. Exposure results using both methods are shown in the tables below.

Table 8. Estimated Exposure to Bonolive® (mg/day) using a 100% Presence Probability Factor

Population Group	Age (yrs.)	M/F	Food Consumers							
			n	% Total	Daily Average Exposure mg/day				90th % RSE	90th Lifetime Exposure mg/day
					Mean	Mean SE	90th	90th SE		
Children	2–11	M	709	97.9	606.5	24.3	1103.2	61.6	5.6	977.0
		F	701	97.4	470.7	16.6	855.8	40.0	4.7	737.7
Teenagers	12–19	M	536	96.1	904.1	83.6	1734.0	160.4	9.3	1533.7
		F	555	96.1	613.8	31.7	1122.7	105.0	9.4	924.1
Adults	20+	M	1961	93.9	887.7	22.7	1733.3	57.6	3.3	1491.1*
		F	2256	95.5	766.9	21.8	1526.3	57.5	3.8	1288.0
Total M/F	2+	M	3206	94.7	850.1	20.9	1681.5	45.5	2.7	1439.3*
		F	3512	95.8	712.7	16.7	1400.2	41.6	3.0	1185.5
Total population	2+	Both genders	6718	95.3	779.2	13.1	1553.2	38.0	2.4	1315.9*

SE = standard error; RSE = relative standard error (<25% is considered reliable).

*Creme warning -2048 (number of days per person should be constant for a foods calculation).

Table 9. Estimated Exposure to Bonolive® Relevant to Body Weight (mg/kg bw/day) using a 100% Presence Probability Factor

Population Group	Age (yrs.)	M/F	Food Consumers							
			n	% Total	Daily Average Exposure mg/kg bw/day				90th % RSE	90th Lifetime Exposure mg/kg bw/day
					Mean	Mean SE	90th	90th SE		
Children	2–11	M	709	97.9	24.0	1.1	46.7	5.2	11.1	40.7
		F	701	97.4	18.9	0.7	34.1	2.3	6.7	31.0
Teenagers	12–19	M	536	96.1	13.1	1.1	25.3	3.3	13.0	22.4
		F	555	96.1	9.7	0.4	18.0	0.7	3.9	15.1
Adults	20+	M	1961	93.9	10.3	0.3	20.3	0.9	4.4	17.7*
		F	2256	95.5	10.3	0.3	20.7	0.6	2.9	17.5
Total M/F	2+	M	3206	94.7	12.6	0.3	25.0	0.9	3.6	22.7*
		F	3512	95.8	11.4	0.3	21.9	0.6	2.7	19.8
Total population	2+	Both genders	6718	95.3	11.9	0.2	23.2	0.5	2.2	21.2*

SE = standard error; RSE = relative standard error (<25% is considered reliable).
*Creme warning -2048 (number of days per person should be constant for a foods calculation).

Table 10. Estimated Exposure to Bonolive® (mg/day) using a 20% Presence Probability Factor

Population Group	Age (yrs.)	M/F	Food Consumers							
			n	% Total	Daily Average Exposure mg/day				90th % RSE	90th Lifetime Exposure mg/day
					Mean	Mean SE	90th	90th SE		
Children	2–11	M	446	61.1	200.5	12.2	419.9	46.1	11.0	233.7
		F	432	60.4	154.8	8.0	266.2	28.4	10.7	210.3
Teenagers	12–19	M	313	57.3	254.4	15.4	515.9	58.4	11.3	373.6**
		F	301	51.2	249.7	34.2	475.1	169.9	35.8*	334.6
Adults	20+	M	1052	52.7	324.9	14.6	612.8	47.0	7.7	447.5
		F	1244	54.2	264.1	17.8	500.2	25.8	5.2	375.2
Total M/F	2+	M	1811	54.4	297.3	10.6	563.4	25.7	4.6	443.8
		F	1977	54.7	247.5	14.4	483.7	26.8	5.5	368.7
Total population	2+	Both genders	3788	54.6	271.7	8.7	523.1	16.2	3.1	412.4

SE = standard error.
*RSE = relative standard error (<25% is considered reliable, >25% is considered unreliable).
**Creme warning -32 (Fourth moment of usual intakes less than 3.0).

Table 11. Estimated Exposure to Bonolive® Relevant to Body Weight (mg/kg bw/day) using a 20% Presence Probability Factor

Population Group	Age (yrs.)	M/F	Food Consumers							
			n	% Total	Daily Average Exposure mg/kg bw/day				90th % RSE	90th Lifetime Exposure mg/kg bw/day
					Mean	Mean SE	90th	90th SE		
Children	2–11	M	446	61.1	7.8	0.4	15.8	1.1	7.0	9.9
		F	432	60.4	6.3	0.3	12.1	0.8	6.6	8.5
Teenagers	12–19	M	313	57.3	3.8	0.2	7.2	0.6	8.3	5.6**
		F	301	51.2	3.8	0.4	7.7	1.0	13.0	5.3**
Adults	20+	M	1052	52.7	3.7	0.2	7.2	0.4	5.6	5.5
		F	1244	54.2	3.6	0.3	6.8	0.4	5.9	4.5
Total M/F	2+	M	1811	54.4	4.4	0.1	8.8	0.4	4.5	7.0
		F	1977	54.7	4.0	0.2	7.8	0.5	6.4	6.0
Total population	2+	Both genders	3788	54.6	4.2	0.1	8.5	0.3	3.5	6.5

SE = standard error; RSE = relative standard error (<25% is considered reliable).
**Creme warning -32 (Fourth moment of usual intakes less than 3.0).

According to the estimates above, approximately 95.3% and 54.6% of the U.S. total population were identified as potential consumers of Bonolive® from the proposed food uses, depending on whether 100% or 20% presence probability was assumed. The 90th percentile aggregate lifetime estimated exposure level for the total population using a 20% presence probability factor was 412.4 mg/day (absolute) and 6.5 mg/kg bw/day (relative to body weight), as shown in the tables above. With regard to individual population groups, the highest absolute lifetime exposure estimate using a 20% presence probability factor was that for adult males (20 years and older) at the 90th percentile, at 447.5 mg/day. The highest exposure estimates relative to body weight at the 90th percentile was that for males aged 2–11, at 9.9 mg/kg bw/day (equivalent to a maximum of approximately 5.4 mg/kg bw/day oleuropein).

As olive products are routinely consumed in the United States, the above exposure estimates are in addition to baseline levels of olive polyphenols in the diet. While oleuropein is abundant in unprocessed olive leaves and fruit, a higher concentration of hydroxytyrosol and tyrosol may be found in the fruit and in olive oil, due to chemical and enzymatic reactions that occur in the plant during maturation of the fruit and also during the processing and fermentation of olives and olive oil, which cause oleuropein to hydrolyze to tyrosol and hydroxytyrosol, thus the latter two compounds are more abundant in the oil.[8,10,11,34-36]

Intake levels of olive oil have been estimated at 0.1–16.3 kg/year per capita; the highest consumers are found in Greece, followed by Spain and Portugal.[37] The

United States was reported to have consumed approximately 0.9 kg/year per capita in 2013. In 2021, domestic olive oil consumption in the United States equals to approximately 406,000 metric tons; 1.22 kg/year per capita[38]. As mentioned previously, oleuropein in olive oil ranges from 0.005–0.12%,[5,9] thus the per capita consumption rate of oleuropein in the United States would be a maximum of approximately 1.4 g oleuropein/year, while in Greece it would be a maximum of 24.1 g oleuropein/year. Compared to the exposure estimates from Bonolive®, the baseline level of consumption of oleuropein from olive oil is considered essentially negligible. Zoidou et al. measured oleuropein and hydroxytyrosol levels in a number of commercial table olive products.[39] They found that oleuropein levels were very low or non-detectable in most of the products. However, they were up to 1.23 mg per olive fruit in a particular black olive product called Throuba Thassos, which is processed using dry salt in a traditional Greek way. Hydroxytyrosol levels were much higher overall in the olive products, at up to 2.05 mg/fruit in kalamata olives. The authors estimated an exposure of 20–40 mg of hydroxytyrosol or 25 mg of oleuropein from the consumption of approximately 20 olives per meal (for oleuropein, the consumption would have to be specifically from Throuba Thassos olives, as other olive types would lead to negligible oleuropein consumption levels). The PRIMED study found consumption of an estimated 25.8 ± 39.2 mg/day of polyphenols such as tyrosols, ligstroside, 3,4-DHPEA-EDA, oleuropein, and 3,4-dihydroxyphenoylglycol by the Spanish participants, obtained from olives and olive oils.[40]

Part 4: Self-limiting Levels of Use

As Bonolive® is not perfectly water soluble, leaving small sediments, it will have self-limiting levels of use in clear beverages. The cost of this specialty brand ingredient will also self-limit the ingredient to some degree. More importantly, oleuropein is well-known to be an extremely bitter molecule, as is true for most polyphenolic compounds.[41-43] Thus use at relatively high concentrations must be combined with ways to mask the unpleasantly bitter flavor—a task that can be difficult to achieve.[43] The taste challenges of integrating oleuropein into functional foods have been discussed in the literature.[41,43] For example, Kranz et al. studied bitterness detection and recognition thresholds of olive leaf extract polyphenols in commercial fruit smoothies using a trained sensory panel.[43] An olive leaf extract containing 40% oleuropein was utilized for the tests. The panelists were able to detect levels as low as 5.8 mg of oleuropein in 100 g of smoothie (58 ppm). In a second step of the study, bitter taste masking of olive leaf extract-enriched fruit smoothies was investigated using the addition of three food ingredients (sucrose, sodium cyclamate, and sodium chloride) at different concentrations. At higher polyphenol levels of 20 mg/100 g (200 ppm), sodium cyclamate and sucrose were able to reduce bitter taste perception by 39.9% and 24.9%, respectively, whereas sodium chloride could not effectively mask bitterness.

Note that the detectable concentration of oleuropein referred to above was much lower than the maximum intended use concentrations for Bonolive®, highlighting those organoleptic effects may indeed be self-limiting for this ingredient. BioActor has experienced challenges overcoming the bitter taste of Bonolive® in working with companies interested in adding their ingredient to various foods. In a number of cases, only much lower levels than the maximum intended use levels stated in this dossier could be utilized due to taste issues, and in other cases, the bitterness proved too challenging to utilize in a particular food at any concentration.

Part 5: Experience Based on Common Use in Food Prior to 1958

The GRAS conclusion for Bonolive® is based on scientific procedures, and thus, experience based on common use in food prior to 1958 is not considered pivotal information. To the best of our knowledge, Bonolive® was not commonly used in foods prior to 1958.

Part 6: Narrative

6.1 Safety Assessment

6.1.1 Pharmacokinetics

Phenolic compounds from virgin olive oil have been shown to be highly bioavailable.[8] Absorption and metabolism of phenolic compounds from olive leaves are also relatively rapid, as is renal clearance.[11,44] Results from several studies suggest that secoiridoid derivatives are hydrolyzed in the upper gastrointestinal tract.[10,25,45,46] Colonic microflora likely also play a role in biotransformation of the phenolic compounds.[46] Numerous metabolites of olive polyphenols have been identified in plasma and urine (over 80 total). The metabolites tend to be identified as conjugated forms (mainly sulfonated and glucuronidated), suggesting extensive first-pass intestinal/hepatic metabolism of these compounds.[25,26,45,47,48]

The oral bioavailability of 250 mg of Bonolive® was studied in healthy pre- and post-menopausal women (eight per group) in a parallel trial to compare the results in these two populations.[25] The pre-menopausal women were all taking monophasic oral contraceptives, and the post-menopausal women had passed menopause by at least 2 years. Bonolive® metabolites were analyzed in plasma and urine over 24 hours using high performance liquid chromatography coupled to electrospray ionization-quadrupole time of flight mass spectrometry (HPLC-ESI-QTOF) and ultra-performance liquid chromatography tied to electrospray triple quadrupose mass spectrometry (UPLC-ESI-QqQ). The majority of the identified metabolites were, as expected, in conjugated form—mainly glucuronidated and sulfated. They appeared rapidly in the plasma; the maximum peak concentration occurred within the first 35–75 minutes. In both groups, the first metabolite to reach the maximum peak concentration was hydroxytyrosol glucuronide. The authors state that the results support the hypothesis that secoiridoid derivatives are hydrolyzed in the upper gastrointestinal tract, since hydroxytyrosol glucuronide appeared rapidly in the plasma. The absorption patterns of the different phenolic compounds in plasma and urine were similar in both groups of women. Plasma levels of hydroxytyrosol glucuronide, hydroxytyrosol sulfate, oleuropein aglycon glucuronide and oleuropein aglycon derivative 1 were higher in post-menopausal women ($p < 0.05$), and these women also excreted fewer sulfated metabolites compared to pre-menopausal women. The vast number of metabolites detected suggests that oleuropein is extensively metabolized in the body. A maximum urine excretion rate was reached in the first four hours, followed by a fast decrease toward baseline levels. The exception was for the sulfated metabolites, the excretion of which was not complete by 24 hours (the time limit of the study). Urine excretion kinetics were similar for the majority of compounds. 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 the pre- and post-menopausal groups. A plasma antioxidant effect was also noted.

In order to quantify the bioavailability and metabolism of oleuropein and hydroxytyrosol from another olive leaf extract, nine healthy volunteers (four females, five males) were given a single low dose (containing 51.1 mg oleuropein and 5.4–9.7 mg hydroxytyrosol) and a high dose (containing 76.6 mg oleuropein and 8.1–14.5 mg hydroxytyrosol) extract as capsules or liquid, with a one-week washout period between.[11,49] In other words, subjects received the opposite strength but the same formulation one week apart. Phenolic content was analyzed in plasma and urine samples over 24 hours using liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS). Conjugated metabolites of hydroxytyrosol (sulfated and glucuronidated) were the primary metabolites identified, comprising 96–99% 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 23–93 minutes). Peak oleuropein concentrations in plasma were notably 6-fold higher following ingestion of liquid versus capsule preparations ($p=0.004$). Males displayed greater plasma area under the curve for conjugated hydroxytyrosol ($p=0.048$). The majority of metabolite recovery occurred within eight hours of ingestion. There was marked inter-individual variation in the results, possibly due to differences in human enzymatic activity.

The absorption of olive oil polyphenols was also investigated in eight healthy ileostomy subjects.[10] Phenols are degraded by microorganisms in the colon, thus if researchers only analyze fecal excretion, it can lead to overestimation of absorption. This is the reason that ileostomy subjects (i.e., subjects without colons) were chosen for the study. The authors also measured urinary excretion in these subjects along with 12 healthy subjects that had a functional colon. Subjects consumed three different supplements containing 100 mg of olive oil phenols with breakfast on separate days in random order. The study was a cross-over design with a one-week washout period between consumption of each supplement, in which no intake of olives or olive oil was allowed. Ileostomy subjects consumed a supplement with mainly nonpolar phenols (e.g., oleuropein- and ligstroside-aglycones; as an ethanolic extract of olive oil), another supplement with mainly polar phenols (e.g., hydroxytyrosol and tyrosol; as a reverse osmosis extract of olive oil), and a third supplement containing 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. The subject/supplement groups are also shown in the table below for clarity:

Supplement	Subjects
Nonpolar	Ileostomy
	Functional colon
Polar	Ileostomy

	Functional colon
Oleuropein-glycoside	Ileostomy
Placebo	Functional colon

Ileostomy effluent/stool and urine were collected for 24 hours after supplement intake. Phenol concentrations were measured using HPLC. Tyrosol and hydroxytyrosol concentrations were low (<4 mol/100 mol of intake) in the ileostomy effluent, and no aglycones were detected. Absorption was confirmed by the excretion of approximately 5–6 mol/100 mol tyrosol and hydroxytyrosol in urine from both subject groups that consumed the polar supplement, 6–12 mol/100 mol after consuming the nonpolar supplement, and ileostomy subjects excreted 16 mol/100 mol (mainly as hydroxytyrosol) after consuming the oleuropein-glycoside supplement. Oleuropein and ligstroside-aglycones were not measured. The authors estimated that up to 66% of the phenols from the nonpolar supplement were absorbed, and the percentage was higher for the polar supplement and the oleuropein-glycoside. Most, if not all of the polyphenols are absorbed in the small intestine.

Differing conditions, such as drying (hot air versus freeze-drying) and extracting (conventional versus ultrasound-assisted) of olive leaves did not have a significant influence on polyphenolic behavior/bioaccessibility during digestion using an in vitro digestion model.[50] The authors found that oleuropein and verbascoside levels were nearly negligible after digestion due to their instability, while luteolin-*7-O*-glucoside was fairly resistant to digestion.

The mechanism of absorption of olive oil phenolics is not clearly defined, 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.[8,25]

The bioavailability, metabolism and distribution of olive phenolic compounds were studied in Wistar rats using an ultra-performance liquid chromatography-tandem mass spectrometer (UPLC-MS/MS).[6] Rats were given a single dose of olive cake (the main by-product of olive oil extraction), containing phenolic compounds typically found in olive oil, including phenyl alcohols, phenolic acids, secoiridoid derivatives, lignans, and flavonoids. Overall, results showed a wide distribution of phenolic compounds and their metabolites (mainly sulphated and glucuronidated conjugate forms) to essentially all tissues in the body, and there was evidence that they crossed the blood brain barrier. Levels 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. Oleuropein derivatives were present in most tissues analyzed after one hour, with an average C_{max} reached at two hours. The main detoxification route was via the kidneys.

When studied on its own, 95% pure oleuropein (extracted from olive leaves) was degraded in gastric aspirates collected from individuals in the fasted state (although degradation products were not specifically quantified, after four hours of incubation in the fasted state, 8.6% of oleuropein content had been transformed to hydroxytyrosol).[51] In the fed state (individuals were fed with 500 mL of Ensure Plus), oleuropein was found to be stable in gastric aspirates but was partially degraded in small intestinal aspirates. All degradation in the study appeared to occur with zero-order kinetics. Oleuropein has also been shown to be converted into hydroxytyrosol at various rates by lactic acid bacterial strains under aerobic and anaerobic conditions,[52] although oleuropein added to milk and yogurt at levels of 0.1–0.4 mg/mL was not affected by heat processing nor lactic acid bacteria in the products.[53]

6.1.2 Toxicology Studies

Genotoxicity and repeated dose oral toxicity studies were conducted to investigate the safety of Bonolive®, in accordance with OECD protocols. These studies were published in the International Journal of Toxicology in 2015.[54]

A bacterial reverse mutation (Ames) test was conducted in compliance with the following internationally accepted guidelines: [1] OECD Guidelines for Testing of Chemicals, No. 471 (adopted 21 July 1997); [2] Commission Regulation (EC) No 440/2008 B13/14 (adopted May 30, 2008); [3] EPA Health Effects Test Guidelines, OPPTS 870.5100 (August 1998), and [4] ICH Guidance S2(R1) (June 2012).

A chromosomal aberration test was conducted in compliance with the following internationally accepted guidelines: [1] OECD Guidelines for Testing of Chemicals, No. 473 (adopted 21 July 1997); [2] EPA Health Effects Test Guidelines, OPPTS 870.5375 (August 1998); and [3] Commission Regulation (EC) No. 440/2008 B 10 (adopted 30 May 2008).

A mammalian erythrocyte micronucleus test was conducted in compliance with the following internationally accepted guidelines: [1] OECD Guidelines for Testing of Chemicals, No. 474 (adopted 21 July 1997); [2] Commission Regulation (EC) No 440/2008, B.12 (adopted 30 May 2008); and [3] EPA Health Effects Test Guidelines, OPPTS 870.5395 (August 1998).

A 14-day repeated-dose oral toxicity study in rats was performed and followed the test procedure recommendations of [1] the OECD Guidelines for the Testing of Chemicals, No. 407 (adopted 03 October 2008) and [2] the US FDA Redbook 2000, IV.C.3.a. (November 2003).

A 90-day repeated-dose oral toxicity study (including a 28-day satellite group) in rats was performed and followed the test procedure recommendations of [1] the

OECD Guidelines for the Testing of Chemicals, No. 408 (adopted 21 September 1998) and [2] the US FDA Redbook 2000, IV.C.4.a. (November 2003).

All five studies were conducted in Good Laboratory Practice (GLP) certified facilities (Toxi-Coop Zrt., Hungary) and in compliance with GLP according to Hungarian GLP regulations, Joint Decree No 9/2001 (III. 30). The Institutional Animal Care and Use Committee (IACUC) of Toxi-Coop Zrt. permitted the conduct of the animal studies according to Standard Operating Procedures (SOP) for animal protection. Additionally, care and use of study animals were in accordance with the National Research Council Guide for Care and Use of Laboratory Animals 8th Edition (published 2011) and in compliance with the principles of the Hungarian Act 2011 CLVIII (modification of Hungarian Act 1998 XXVIII) regulating animal protection. The studies are described in the summaries below.

Bacterial Reverse Mutation Assay[54]

Purpose: To evaluate the mutagenic potential of Bonolive®.

Methods: Four strains of *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537) and one strain of *Escherichia coli* (WP2 *uvrA*) were used in the presence and absence of rat liver S9 metabolic activation with appropriate positive and negative controls. The study included a preliminary solubility test, a preliminary range-finding test, an initial mutation test (IMT; plate incorporation assay) and a confirmatory mutation test (CMT; pre-incubation assay). Concentrations of Bonolive® used for the IMT and CMT were based on the preliminary results and were as follows: 51.2, 128, 320, 800, 2000 and 5000 µg/plate.

Results: Spontaneous revertant colony numbers of the vehicle control agreed with historical control data, and positive controls induced the expected responses. No biologically relevant increases were seen in revertant colony numbers of any of the five bacterial strains upon treatment with Bonolive® at any of the concentration levels either in the presence or absence of an S9 activation system.

Conclusions: Under the experimental conditions applied, Bonolive® was considered non-mutagenic at concentrations up to the maximum recommended test concentration of 5000 µg/plate.

Chromosomal Aberration Study[54]

Purpose: To evaluate the clastogenic potential of Bonolive®.

Methods: Bonolive® was dissolved in Dulbecco's Modified Eagle's medium (DME) medium, and the concentrations listed below were chosen on the basis of preliminary cytotoxicity investigations. The chromosomal aberration assay was conducted in two independent experiments (each in duplicate) using V79 Chinese

hamster lung cells. The cells were exposed to the negative control or each test article concentration with and without metabolic activation using rat liver preparations (S9-mix). Groups of cells were also exposed to the respective positive controls for use with or without S9-mix. Exposure and sampling times were as follows:

- Experiment A: 3h treatment with and without S9-mix/20h sampling time.
    - Without S9: 250, 500, 750 and 1000 µg/mL
    - With S9: 250, 500, 750, 1000 and 1250 µg/mL
- Experiment B: 20h treatment without S9-mix/20 and 28h sampling times.
    - Without S9: 62.5, 125, 250 and 500 µg/mL
- Experiment B: 3h treatment with S9-mix/28h sampling time.
    - With S9: 500, 750, 1000, 1250 and 1500 µg/mL

Following treatment (exposure) and sampling (expression) time, cells were exposed to colchicine (0.2 µg/mL) 2–3 hours prior to harvesting and fixing for slide preparation. Chromosome aberration frequencies were then scored blind for at least 200 well-spread metaphase cells.

Results: In both experiments, A and B, no statistically significant differences between treatment and negative (solvent) control groups and no dose-response relationships were noted. No increase in the rate of polyploidy and endoreduplicated metaphases were observed after treatment with the different concentrations of Bonolive® with or without metabolic activation. Positive controls induced biologically and statistically significant increases in the number of cells with chromosome aberrations over background.

Conclusions: Bonolive® did not induce structural chromosome aberrations and is not considered clastogenic in this test system.

***Micronucleus Study*[54]**

Purpose: To evaluate the in vivo mutagenic potential of Bonolive®.

Methods: A single dose of Bonolive® was administered by gavage to male Crl:NMRI BR mice at test concentrations of 0 (vehicle control), 500, 1000 and 2000 mg/kg bw. The negative control/vehicle was Humaqua. The positive control, cyclophosphamide 60 mg/kg bw, was administered by intraperitoneal injection. All treatments were administered at a uniform volume of 10 mL/kg bw. The negative control and high-dose groups consisted of 10 animals, and all other groups consisted of five animals. The main micronucleus test was conducted at the doses described above in males only based on the results of a preliminary toxicity test that was conducted using a single dose of Bonolive®, by gavage, at a concentration of 2000 mg/kg bw in two animals/sex/group in order to determine the high-dose and assess

gender differences. No mortality, signs of toxicity or gender-specific effects were observed in the preliminary test.

Group designation:

Dose (mg/kg bw/day)		No. of Males
Negative Control	0	10
Low–dose	500	5
Mid–dose	1000	5
High–dose	2000	10*
Cyclophosphamide	60	5

*Two additional males were dosed in the high-dose group to replace any which might have died before the end of the study, however no deaths occurred.

In the low and mid-dose groups, the sampling from bone marrow was performed once at 24 hours after treatment and twice, at 24 and 48 hours after treatment, in the high dose and negative control groups. The positive control animals were sampled only at 24 hours post-treatment. Five animals per dose group were used on each occasion. Two thousand polychromatic erythrocytes (PCEs) per animal were scored for frequency of micronuclei.

Results: No mortality was observed the study. On the day of treatment, a slight decrease in activity and piloerection were observed in four of the 10 male mice treated with 2000 mg/kg bw of Bonolive®. These symptoms were not observed at 24 and 48 hours after treatment. Because no mortality occurred, bone marrow slides were not prepared for the two extra animals included in the high-dose group. No significant differences were observed in frequency of micronucleated PCEs (MPCEs) or proportion of PCE to mature erythrocytes between the three dose groups compared to the negative control, and all results were within the laboratory's historical control range. A large, statistically significant increase in MPCE frequency was observed in the positive control group compared to negative control.

Conclusions: Bonolive®, at concentrations up to the limit dose of 2000 mg/kg bw, did not show any genotoxic activity in the mouse micronucleus test.

Fourteen-day Repeated-Dose Oral Toxicity Study[54]

Purpose: To obtain information on the toxic potential and to evaluate the maximum tolerated dose of Bonolive® in male and female rats from repeated exposure to the test article via gavage over a 14-day repeated dose test period.

Methods: Five groups of five SPF Crl:(WI)BR Wistar rats/sex/group were administered Bonolive® (formulated in a 1% Tween 80 vehicle) at concentrations to provide for uniform administration by gavage of a dose volume of 10 mL/kg bw; doses were 0 (vehicle-control), 300, 600, 1000 or 2000 mg/kg bw/day for 14 days.

Animals were observed for mortality twice a day, and detailed clinical observations were performed daily after treatment. Body weights were recorded twice weekly. Food consumption was determined weekly to coincide with body weight measurements during the study. Ophthalmologic examinations were performed on all animals before the first treatment and during the last week. Clinical pathology and gross pathology examinations were conducted on all animals one day after the last treatment. Selected organs were weighed. Full histopathological examinations were performed on all animals of the control and high dose groups and gross lesions of animals of the low and mid-dose groups (including the testes and epididymides of one animal in the 1000 mg/kg bw/day group). Kidneys of animals in the 300, 600 and 1000 mg/kg bw/day groups were also assessed histologically due to findings in the high-dose animals.

Results: There was no mortality during the course of the study. Toxic signs related to the test article were not found during the detailed clinical observations. No test article related to body weight, or body weight gain changes were observed. Mean daily food consumption and feed efficiency were not influenced by the test article. There were no test articles related to eye alterations or pathologic changes in hematological or clinical chemistry parameters. Specific macroscopic alterations related to the test article were not found during the terminal necropsy, and no test article-related changes in organ weights were noted. One male animal from the 1000 mg/kg bw/day group was missing the head of one epididymis (congenital absence). Histopathological evaluation of organs revealed hyaline-like droplets in the kidneys of male animals of 1000 and 2000 mg/kg bw/day group animals. The incidence and severity of the lesions were less in the 1000 mg/kg bw/day group than in the 2000 mg/kg bw/day group.

Discussion and Conclusions: Oral administration of Bonolive® was associated with renal changes (hyaline-like droplet nephropathy) in male rats in the 1000 and 2000 mg/kg bw/day doses. There were no additional treatment-related findings in male or female rats after 14-day oral administration at 300 or 600 mg/kg bw/day or in female animals of the 1000 or 2000 mg/kg bw/day doses.

Hyaline droplet nephropathy describes a spectrum of morphologic changes in the kidneys of male rats induced by a variety of compounds and conditions and may not be relevant to humans.[55-57] There is generally an abnormal accumulation of α-2μ-globulin phagolysosomes of the tubular epithelium in this condition. The finding is common in male rats and is not seen in humans although occasionally its severity can occur in a dose-related manner after administration of a test article, suggesting a possible effect. One proposed mechanism of interaction is that a chemical or metabolite may bind with α-2μ-globulin or alter its structure so that the tubular cell lysosomal enzymes cannot degrade the protein complex. Other proposed mechanisms include direct cytotoxic effects.[58] It is unlikely that the various chemicals associated with hyaline droplet nephropathy in the male rat throughout the literature act by the same mechanism. Some chemicals that produce hyaline

droplet nephropathy in male rats also produce renal toxicity (unassociated with α-2μ-globulin) in female rats, whereas others produce no effects in the kidney of female rats.

Because of this finding in the 14-day study, a satellite study of animals (5 per sex per group) was added to the following 90-day study plan. These satellite animals were terminated on day 28 (as opposed to day 90) to obtain preliminary data, and the kidneys of male animals in all dose groups were processed and examined histopathologically to investigate the possible presence of hyaline-like droplets in the epithelial cells of proximal convoluted tubules before the remainder of the 90-days of exposure in the main groups.

Ninety-day Repeated-Dose Oral Toxicity Study[54]

Purpose: To continue to evaluate the potential health hazards, including identification of toxic effects and target organs, of repeated oral exposure to Bonolive® in male and female rats for 90 days, and to determine a NOAEL.

Methods: Four groups of 20 SPF Crl:(WI)BR Wistar rats (10 per sex per group) were administered Bonolive® dissolved in 1% Tween 80 (vehicle) at concentrations to provide for uniform administration by gavage of a dose volume of 10 mL/kg bw. Doses were 0 (vehicle-control), 360, 600 and 1000 mg/kg bw/day, given for 90-days. One additional female animal was added to the study on Day 2 to replace a female animal that died very early on in the 1000 mg/kg bw/day group. Individual data of this animal was reported but was not included in the overall evaluation.

To help determine the significance and repeatability of the hyaline-like droplet findings noted in the 14-day study, a 28-day satellite group (five animals per sex per group) was added to the study for early histopathological examination with a specific focus on nephropathy. The continuation with the 90-day study plan would be dependent upon the findings of the 28-day satellite group.

All animals were observed for mortality twice a day during the course of the study. General clinical observations were performed daily after treatment. Detailed clinical observations were made on all animals weekly. A functional observation battery was conducted during the last week of the treatment. Body weight was recorded twice weekly during weeks 1–4 and once weekly thereafter (weeks 5–13). Food consumption was determined weekly to coincide with body weight measurements. Ophthalmologic examinations were performed on all animals before the first treatment and on animals of the control and high dose groups during the last week of treatment. Clinical pathology and gross pathology examinations were conducted on all animals one day after the last treatment (i.e., animals in satellite groups on day 28, animals of main groups on day 90 (males) and on day 91 (females). Selected organs were weighed. Full histopathological examinations were performed on all animals of the control and high dose groups. Kidneys of male animals in the satellite

groups at 360 and 600 mg/kg bw/day were also processed histologically. In the low and middle dose groups, organs with any other macroscopic findings were processed and examined histologically. All quantitative data was subjected to statistical analysis.

Results: There was no test article-related mortality in any of the satellite or main groups. One female and one male animal from the 1000 mg/kg bw/day died during the study, both deemed due to gavage procedure, on Day 2 and Day 60, respectively. Full examinations were performed on the animals on the day of their death. For the female animal, there were no preceding clinical signs or gross pathology findings, and histopathological examination revealed acute catarrhal pneumonia and serous-fibrinous pleuritis. For the male animal, death was preceded by salivation, convulsion, prone positioning, decreased activity, dyspnea, and narrow eye aperture, all of which occurred shortly after treatment. Gross pathology revealed dark red lungs, dark red liver, and dark color of the right lobe of the thymus and yellowish fluid content in the thoracic cavity in full compliance with histopathological findings of acute alveolar emphysema accompanied by acute hemorrhage in the lungs and congestion of the liver and thymus. There were no histopathologic findings related to the kidneys.

As further described below, no toxicologically relevant findings were noted in the satellite animals after 28-days, including no gross or histopathological findings related to the kidneys. Thus, the full 90-day study plan was carried out.

Toxic signs related to the test article were not found during daily or detailed weekly observations. Several common findings occurred with low incidence in both the control and low or mid dose groups but not in the high dose group, and thus were not considered toxicologically relevant (e.g., slight salivation in some animals, and some individual dermal clinical signs). The functional observation battery did not reveal any test article influence on animal behavior or neurological functioning.

No test article related body weight or body weight gain changes were observed in satellite or main groups. Statistically significant differences with respect to the control were noted for the lower mean body weight gain of female animals in the 1000 mg/kg bw/day group between Days 11–14, Days 28–35 and Days 56–63, but did not result in changes to mean body weight or in the total body weight gain compared to controls. Therefore, these transient differences were not considered biologically or toxicologically relevant. Mean daily food consumption was not influenced by the test article. There were several sporadic statistically significant differences in feed efficiency with respect to controls in the main group which were considered normal biological variation (male animals in the 360 and 1000 mg/g bw/day groups were slightly lower than controls between Days 28 and 35, as were female animals in the 600 mg/kg bw/day group between Days 0 and 7).

There were no treatment-related eye alterations in any of the groups, nor any toxicologically relevant changes in the evaluated hematology, blood coagulation or

clinical chemistry parameters at the end of the 28-day or 90-day treatment periods. Some sporadic, statistically significant findings that were not considered toxicologically relevant are shown in the tables below.

Table 12. Selected Hematological Findings in the 90-Day Repeated Oral Toxicity Study[54]

Group	NEU	LYM	MONO	RBC	HGB	HCT	PLT	APTT
(mg/kg bw/d)	%	%	%	$x10^{12}$/L	g/L	L/L	$x10^{9}$/L	sec
Male (Satellite groups n=5 each)								
Control	9.76 ± 2.55	86.48 ± 3.03	2.58 ± 0.66	8.99 ± 0.22	170.80 ± 2.39	0.48 ± 0.01	987.00 ± 104.03	16.54 ± 2.18
360	15.28 ± 1.36*	79.90 ± 2.50*	3.12 ± 0.42	8.49 ± 0.27*	162.40 ± 4.67*	0.45 ± 0.01*	909.80 ± 97.87	16.22 ± 2.56
600	18.04 ± 3.31**	76.98 ± 3.73**	3.82 ± 0.81*	9.16 ± 0.36	172.00 ± 6.04	0.48 ± 0.2	975.00 ± 157.82	17.12 ± 2.03
1000	11.34 ± 4.20	83.76 ± 5.07	2.90 ± 0.66	8.73 ± 0.46	166.60 ± 7.60	0.46 ± 0.02	905.00 ± 135.94	15.78 ± 0.96
Historical Range‡	6.0–39.8	54.4–91.5	0.3–5.1	6.72–9.83	124–179	0.354–0.489	625–1173	13.1–22.9
Male (Main groups n=10 each, except 1000 mg/kg n=9)								
Control	14.87 ± 2.18	79.14 ± 2.29	4.03 ± 0.61	9.76 ± 0.42	166.20 ± 7.30	0.45 ± 0.02	935.60 ± 117.62	18.28 ± 2.05
360	18.63 ± 5.24	73.38 ± 5.90	5.63 ± 1.32**	9.75 ± 0.34	168.60 ± 5.19	0.46 ± 0.01	900.80 ± 113.87	19.36 ± 1.64
600	18.47 ± 6.72	74.81 ± 7.50	4.88 ± 1.03	9.82 ± 0.45	168.70 ± 6.93	0.46 ±0.02	1055.60 ± 145.35	19.65 ± 3.09
1000	18.02 ± 6.45	76.20 ± 7.03	4.13 ± 1.26	9.76 ± 0.35	168.00 ± 6.93	0.46 ± 0.02	1034.11 ± 128.93	19.26 ± 1.63
Historical Range‡	8.9–24.6	67.7–86.8	1.4–6.3	8.61–10.61	155–183	0.416–0.500	792–1349	14.3–23.1
Female (Satellite groups n=5 each)								
Control	17.60 ± 4.49	77.64 ± 3.34	3.12 ± 0.96	8.85 ± 0.21	164.60 ± 8.26	0.45 ± 0.02	842.60 ± 126.36	18.88 ± 1.50
360	15.10 ± 2.06	79.64 ± 2.92	3.12 ± 0.72	8.71 ± 0.13	159.60 ± 5.55	0.44 ± 0.02	898.80 ± 127.65	17.42 ± 1.50
600	17.78 ± 7.01	79.16 ± 6.94	1.90 ± 0.34*	8.61 ± 0.61	162.80 ± 6.30	0.45 ± 0.01	802.80 ± 113.67	18.36 ± 2.16
1000	13.84 ± 5.83	82.36 ± 5.89	2.42 ± 0.65	8.54 ± 0.32	160.60 ± 6.19	0.44 ± 0.02	930.40 ± 161.63	17.12 ± 1.17
Historical Range‡	4.8–25	72.1–93.5	0.3–5.5	7.58–9.35	147–174	0.408–0.476	659–1088	13.9–25.1
Female (Main groups n=10 each)								
Control	13.51 ± 4.76	82.40 ± 5.17	2.44 ± 0.72	9.09 ± 0.54	163.80 ± 7.67	0.45 ± 0.02	802.30 ± 100.73	18.88 ±0.92
360	16.24 ± 7.47	79.83 ± 7.79	2.27 ± 0.73	8.90 ± 0.44	165.00 ± 6.94	0.45 ± 0.02	949.70 ± 98.40**	19.17 ± 1.94
600	18.08 ± 12.56	77.31 ± 14.54	2.74 ± 2.22	9.11 ± 0.78	166.10 ± 11.71	0.46 ± 0.03	854.70 ± 117.95	20.64 ± 1.96*
1000	16.15 ± 3.95	80.40 ± 4.35	2.14 ± 0.98	8.87 ± 0.58	163.90 ± 11.24	0.45 ± 0.03	903.50 ± 122.43	20.17 ± 1.67
Historical Range‡	6.8–28.1	68.4–90.4	0.8–4.5	7.97–9.94	152–176	0.423–0.488	675–1176	12.8–21.9

Data represent the mean values and the standard deviation.

†Only paramteters with statistically significant findings are shown in table.

*$P < 0.05$ and **$P < 0.01$

‡minimum and maximum levels reported as the range of historical control values

Table 13. Selected Clinical Chemistry Findings in the 90-Day Repeated Oral Toxicity Study[54]

Group	ALT	AST	ALP	TBIL	CREA	CHOL	BAC	Pi	Ca++	Na+	Cl-	ALB	TPROT	A/G
(mg/kg bw/d)	U/L	U/L	U/L	μmol/L	μmol/L	mmol/L	μmol/L	mmol/L	mmol/L	mmol/L	mmol/L	g/L	g/L	
Male (Satellite groups n=5 each)														
Control	43.22 ± 6.92	91.66 ±10.18	143.20 ± 15.40	1.98 ± 0.36	25.90 ± 1.07	2.10 ± 0.34	42.12 ± 20.01	3.11 ± 0.26	2.75 ± 0.10	144.60 ± 1.52	103.54 ± 1.50	33.04 ± 0.88	61.16 ± 3.24	1.2 ± 0.1
360	33.04 ± 4.07*	85.62 ± 7.71	132.00 ± 12.35	1.92 ± 0.24	27.72 ± 1.70	1.81 ± 0.32	28.72 ± 9.01	2.98 ± 0.27	2.73 ± 0.08	144.20 ± 0.84	103.56 ± 0.87	32.80 ± 0.83	58.04 ± 0.95	1.3 ± 0.1*
600	40.50 ± 6.73	96.94 ± 15.06	117.20 ± 11.12*	2.10 ± 0.16	26.36 ± 1.70	1.63 ± 0.14*	27.20 ± 5.76	2.76 ± 0.19	2.71 ± 0.10	145.40 ± 1.14	104.74 ± 1.26	33.72 ± 2.19	60.90 ± 4.69	1.2 ± 0.1
1000	36.54 ± 6.34	94.58 ± 20.55	119.40 ± 20.38*	1.90 ± 0.10	25.62 ± 2.54	1.66 ± 0.14*	39.52 ± 12.17	2.83 ± 0.16	2.75 ± 0.05	144.40 ± 2.19	103.78 ± 1.54	33.40 ± 0.62	59.48 ± 1.52	1.3 ± 0.0*
Historical Range‡	34.4–87.8	73.3–130.4	103–290	0.12–2.78	16.6–26.8	1.42–2.60	9.5–131.0	1.98–3.42	2.57–2.92	137–147	96.2–105.5	31.6–35.5	56.1–66.2	1.1–1.5
Male (Main groups n=10 each, except 1000 mg/kg n=9)														
Control	49.35 ± 9.41	112.94 ± 12.05	71.40 ± 14.38	2.36 ± 0.38	31.70 ± 2.60	1.87 ± 0.21	42.58 ± 13.87	2.21 ± 0.12	2.64 ± 0.04	142.10 ± 0.99	104.23 ± 0.67	33.35 ± 0.75	58.60 ± 1.59	1.32 ± 0.09
360	46.05 ± 10.07	104.22 ± 16.18	65.00 ± 11.26	1.98 ± 0.26*	31.20 ± 2.24	1.76 ± 0.35	28.32 ± 6.88*	2.14 ± 0.16	2.67 ± 0.05	141.70 ± 1.25	105.29 ± 0.94*	33.64 ± 0.76	58.19 ± 1.49	1.35 ± 0.07
600	39.01 ± 8.32*	104.31 ± 16.39	56.10 ± 6.47*	2.29 ± 0.25	28.14 ± 1.92**	1.98 ± 0.37	31.44 ± 14.57	2.40 ± 0.23*	2.78 ± 0.06**	140.40 ± 1.17**	103.63 ± 1.14	33.99 ± 1.03	60.05 ± 3.17	1.31 ± 0.11
1000	37.41 ± 4.72**	92.68 ±14.16**	52.56 ± 5.61**	2.21 ± 0.30	27.61 ± 2.18**	1.82 ± 0.26	32.06 ± 15.20	2.34 ± 0.16	2.81 ± 0.08**	140.11 ± 0.93**	102.99 ± 0.88**	34.40 ± 0.79*	59.16 ± 2.43	1.40 ± 0.09
Historical Range‡	41.8–101.6	80.3–160.4	61–133	2.04–3.78	20.8–33.7	1.26–3.09	19.4–108.2	1.71–2.46	2.39–2.84	136–146	96.8–106.3	28.6–35.2	52.1–65.5	1.1–1.5
Female (Satellite groups n=5 each)														
Control	49.22 ± 8.25	106.10 ± 12.86	71.80 ± 5.76	2.64 ± 0.22	32.64 ± 2.83	2.10 ± 0.30	35.32 ± 9.98	2.61 ± 0.42	2.72 ± 0.16	148.20 ± 1.79	107.46 ± 1.77	34.08 ± 0.68	62.14 ± 1.65	1.22 ± 0.08
360	44.46 ± 6.60	115.16 ± 31.04	67.80 ± 14.55	3.33 ± 0.40**	32.42 ± 4.24	2.16 ± 0.21	52.76 ± 24.82	2.58 ± 0.18	2.76 ± 0.12	155.40 ± 3.51**	113.62 ± 2.66**	35.94 ± 0.98*	65.92 ± 1.87*	1.18 ± 0.04
600	32.22 ± 2.96**	99.62 ± 14.90	77.40 ± 12.97	2.80 ± 0.25	30.32 ± 1.82	1.67 ± 0.51	32.42 ± 22.21	2.54 ± 0.42	2.65 ± 0.02	148.40 ± 1.14	108.18 ± 1.18	35.04 ± 1.37	64.80 ± 2.89	1.18 ± 0.04
1000	34.06 ± 5.88**	91.26 ± 5.87	61.20 ± 11.78	2.89 ± 0.12	28.06 ± 4.99	2.12 ± 0.47	67.48 ± 61.53	2.60 ± 0.17	2.79 ± 0.12	146.60 ± 3.29	105.40 ± 2.65	35.12 ± 1.08	62.04 ± 3.36	1.30 ± 0.07
Historical Range‡	30.9–70.2	78.4–121.2	47–171	0.57–2.96	17.3–36.0	1.27–2.69	8.0–62.9	1.32–3.30	2.49–2.89	134–151	98.7–110.3	30.6–38.1	52.1–65.9	1.1–1.6
Female (Main groups n=10 each)														
Control	42.50 ± 11.36	86.60 ± 10.72	34.90 ± 7.43	2.20 ± 0.63	36.69 ± 2.93	2.22 ± 0.45	18.51 ± 5.53	1.39 ± 0.22	2.55 ± 0.07	144.00 ± 2.16	104.02 ± 1.34	35.23 ± 1.99	62.43 ± 4.60	1.29 ± 0.06
360	33.15 ± 5.42**	78.30 ± 14.16	36.90 ± 12.14	2.17 ± 0.51	36.56 ± 4.39	2.17 ± 0.71	31.42 ± 18.02*	1.41 ± 0.27	2.50 ± 0.07	142.40 ± 1.71	104.11 ± 2.15	35.26 ± 1.30	61.23 ± 3.11	1.36 ± 0.1
600	31.39 ± 6.78**	80.03 ± 22.05	30.50 ± 11.63	1.80 ± 0.51	36.40 ± 2.67	1.93 ± 0.48	30.20 ± 20.01	1.41 ± 0.18	2.45 ± 0.06**	140.90 ± 0.57**	103.59 ± 1.00	33.92 ± 2.72	58.99 ± 4.69	1.36 ± 0.07
1000	26.10 ± 5.26**	72.94 ± 8.73*	34.00 ± 6.58	2.27 ± 0.51	34.27 ± 2.09	1.82 ± 0.28	37.97 ± 18.02**	1.46 ± 0.32	2.48 ± 0.07*	141.10 ± 1.66**	104.30 ± 1.06	34.86 ± 1.79	60.39 ± 2.54	1.36 ± 0.08
Historical Range‡	26.9–87.4	82.4–193.6	35–78	1.88–4.49	20.5–40.6	1.26–2.78	13.6–189.4	1.11–2.07	2.22–2.93	120–145	82.4–108.4	27.0–37.1	49.3–70.4	1.0–1.5

Data represent the mean values and the standard deviation.

†Only paramteters with statistically significant findings are shown in table.

*$P < 0.05$ and **$P < 0.01$

‡minimum and maximum levels reported as the range of historical control values

Specific macroscopic alterations indicative of test article effects were not observed in the organs or tissues of animals from any dose group or treatment period; individual macroscopic findings are shown in the table below. Note that hydrometra is a frequent observation in experimental rats, which is related to the female sexual cycle.

Table 14. Necropsy Findings in the Surviving Animals in the 90-Day Repeated Oral Toxicity Study[54]

Group	No findings	Thumus	Kidneys	Uterus
(mg/kg bw/d)		Point-like hemorrhages	Pyelectasia	Hydrometra
Male (Satellite groups n=5 each)				
Control	4 of 5	1 of 5	0 of 5	N/A
360	5 of 5	0 of 5	0 of 5	N/A
600	3 of 5	0 of 5	2 of 5	N/A
1000	5 of 5	0 of 5	0 of 5	N/A
Male (Main group survivors n=10 each, 1 death in 1000 mg/kg group thus n=9)				
Control	10 of 10	0 of 10	0 of 10	N/A
360	10 of 10	0 of 10	0 of 10	N/A
600	7 of 10	0 of 10	3 of 10	N/A
1000	9 of 9	0 of 9	0 of 9	N/A
Female (Satellite groups n=5 each)				
Control	5 of 5	0 of 5	0 of 5	0 of 5
360	4 of 5	0 of 5	0 of 5	1 of 5
600	4 of 5	0 of 5	0 of 5	1 of 5
1000	2 of 5	0 of 5	0 of 5	3 of 5
Female (Main group survivors n=10 each)				
Control	9 of 10	0 of 10	0 of 10	1 of 10
360	8 of 10	0 of 10	0 of 10	2 of 10
600	7 of 10	0 of 10	0 of 10	3 of 10
1000	9 of 10	0 of 10	0 of 10	1 of 10
note: necropsy findings of dead animals are not described here, but rather are described in the text				
N/A = not applicable				

Test article effects were not observed related to organ weights when comparing those from treated animals to those of the control group. Statistical significance was noted for slightly higher mean liver weight relative to body weight in male animals of the 600 and 1000 mg/kg bw/day satellite groups. This effect was also seen in males for liver weight relative to body weight in the 360 and 1000 mg/kg bw/day and in females of the 600 mg/kg bw/day groups in the main study. These changes were of a small degree and corroborative findings were not detected during histopathological examination of the liver; thus, the findings were not considered toxicologically meaningful.

Histopathological investigations did not reveal any test article-related lesions in the high-dose group animals. Alveolar emphysema and hyperplasia of bronchus associated lymphoid tissue (BALT) were detected in the lungs of some male and female animals in satellite and main groups but were seen equally in control and high dose group animals and/or at increased levels in control animals. Hemorrhage was noted for one male animal of the satellite control group in the thymus. Acute pulmonary emphysema and hemorrhages in the thymus were considered consequences of hypoxia, dyspnea and circulatory disturbance that developed during exsanguination. Hyperplasia of BALT is an immunomorphological phenomenon[59,60] that was not considered to have toxicological significance. Dilation of the uterine horns in 3/5 females in the 1000 mg/kg bw/day satellite group, 1/10 in the control and 1/10 in the 1000 mg/kg bw/day main groups was not

considered toxicologically relevant as it is considered a common neurohormonal phenomenon in connection with the proestrus phase of the sexual cycle.[61,62]

Conclusions: Repeated administration by gavage of 360, 600 and 1000 mg/kg bw/day of Bonolive® for 90 days did not cause adverse effects or signs of toxicity in male or female SPF Crl:(WI) BR Wistar rats. Notably, unlike in the 14-day study, no hyaline-like droplet nephropathy was observed in this study in males of the satellite or main groups (including five satellite males sacrificed on day 28, one male that died on day 60, and nine males that were sacrificed on day 90), suggesting that the original finding in the 14-day study may have been due to chance. The NOAEL of the 90-day study was determined to be 1000 mg/kg bw/day for both male and female animals; the highest doses tested.

6.1.3 Additional Scientific Studies

In vitro studies

Qabaha[63] and colleagues evaluated the cytoxicity potential of an ethanolic olive leaf extract and its individual components in vitro. The cytoxic potential was tested using polymorphonuclear cells isolated from human blood. After stimulation with 1 g/mL lipopolysaccharide, polymorphonuclear cells were given olive leaf extract at a dosage of 320 mg/mL for 16 hours. Control cell cultures with or without liposaccharide stimulation were compared to the results. When compared to cell culture with or without lipopolysaccharide stimulation, the olive leaf extract at a concentration of 320g/mL had no significant influence on polymorphonuclear cell viability.

Animal Studies

Kumral and colleagues gave an olive leaf extract to male Sprague-Dawley rats at doses of approximately 500 and 1000 mg/kg/day for 12 days in their drinking water.[64] The extract was approximately 10% oleuropein. On day eight, some of the rats were given doxorubicin, a drug known to increase oxidative stress in several organs. The olive leaf extract led to decreases in serum cardiac troponin I, urea, ALT, and AST compared to animals that were exposed to doxorubicin alone. The extract also ameliorated histopathological findings caused by doxorubicin. Oxidation markers like malondialdehyde, diene conjugate and protein carbonyl were also decreased by olive leaf extract in the heart, hepatic, and renal tissues, while glutathione levels increased compared to the group treated with doxorubicin alone. Thus, it appeared that olive leaf treatment decreased doxorubicin-induced oxidative stress and injury.

An olive pulp extract containing 6% olive polyphenols (HIDROX™, CreAgri, Inc. California) was characterized in a series of published toxicological studies.[65] No

test-article related adverse clinical, hematologic, biochemical, organ weight or gross necropsy findings occurred in a 90-day study in Crl:CD (SD)IGS BR VAF/Plus rats, and the NOAEL was 2000 mg/kg bw, the highest dose tested. Additionally, this dose did not produce adverse effects in a dose-range finding reproduction study or a developmental toxicity study in rats.

Olive leaf extracts have been shown to be protective against the induction of oxidative stress related damage in animal studies. An aqueous extract of olive leaf was able to protect against toxicity associated with seven weeks of treatment with diazinon (an organophosphorus insecticide and a neurotoxin) in mice.[66] Similarly, an extract of olive leaves was able to antagonize permethrin (a widely used chemical for insecticidal and other uses)-induced genotoxic and oxidative toxicity in rats, as well as cultured human blood cells.[67,68] An olive leaf extract was also shown to protect Wistar rats against lead accumulation in the brain, and appears to protect against lead induced brain damage through inhibition of apoptosis, oxidative stress, inflammation and cell metabolism impairments.[69] An ethanolic extract of olive leaves (containing larger amounts of oleuropein, hydroxytyrosol, verbascoside, luteolin, and quercetin compared to a methanolic extract) was able to protect rat cardiomyocytes (better than the methanolic extract or individual phenolic compounds) when using a 4-hydroxynonenal-induced carbonyl stress and toxicity model of oxidative damage.[70]

In an ex vivo study, a dry olive leaf ethanolic extract (standardized to 18–26% oleuropein) was shown to significantly reduce adrenaline and hydrogen peroxide-induced DNA damage of peripheral blood leukocytes from six healthy subjects.[71] It was protective at all concentrations tested (0.125, 0.5 and 1 mg/mL), although it was most effective at the lowest concentration. It was protective when used pre-treatment as well as post-treatment. The results support the notion that olive leaf extract has genoprotective and antioxidant properties.

An olive leaf extract was found to be anticlastogenic in a mouse micronucleus assay when animals were given an x-ray irradiation treatment.[72] In this study, some animals were given the extract orally for five days prior to exposure to irradiation, while other animals were given the extract as a single injection into the gastric lumen 15 minutes post-irradiation. The extract was found to be radioprotective (and consequently, anticlastogenic) both when given prior to and after irradiation.

Twenty-four male and female crossbred growing pigs were randomly assigned to 0, 25 or 50 g/kg olive leaf powder mixed into their pelleted feed.[73] The total polyphenolic level in the diets was 0, 1600 and 3200 mg/kg, respectively. Body weights and feed intake were recorded for the animals throughout the study. At the end of the growing period, venous blood was obtained to assess liver function. Liver, lungs, heart, tongue, perinephric adipose tissue, and kidneys were weighed after slaughter, and samples of longissimus muscle were taken between the lumbar vertebrae. Pigs fed the 50 g/kg feed diet had lower final body weight and daily

weight gain, but a higher feed/gain ratio than those fed the conventional diet. Olive leaf supplementation at 25 g/kg did not affect performance parameters, except for feed/gain ratio. No effects were seen in relative organ weights, and there were no differences in serum levels of AST, ALT, GGT, and ALP, serum lipoprotein profiles, or direct bilirubin serum levels between groups. The authors concluded that olive leaves could be included in pig diets at 25 g/kg to improve meat quality.

In a *Drosophila* wing-spot test, consumption of an olive leaf methanol extract (0.8–12 mg polar phenols/4 mL medium) or pure oleuropein (0.8–8 mg/4 mL medium) led to no significant increase in any type of mutant spot.[74] This test detects various mutational events in vivo, including mitotic recombination.

Guex et al., (2018) performed a study to enhance the already available information regarding the toxicity of olive leaves. The safety of exposure to an ethanolic extract of Olea europaea L. leaves ("EEO") in Wistar rats, for 28 days was assessed. Male and female Wistar rats (weighing 150–200 g) were randomly placed in polypropylene cages according to gender. The animals were acclimatized for a week prior to the beginning of the experiment and kept at a constant temperature (22 2 °C) with a 12-hour light/dark cycle. Food (regular diet) and water were freely available to all animals. With minor adjustments, acute and subacute toxicity tests were conducted according to OECD guidelines 423 and 407, respectively (OECD, 2001, 2008).

For the acute toxicity study, the olive leaf extract was given in three males and three females (n=6) that fasted overnight, in a single dose of 2000 mg/kg via oral gavage (free access to water). A negative control group was created by giving both males and females (n=6) a 51 percent ethanol solution (10mL/kg). The animals' body weight was measured shortly before the provision of the extract was and subsequently daily during the treatment period. Animals were observed individually for the first 30 minutes after administration and then daily for 14 days. Mortality, alterations in skin and fur, eyes, and mucous membranes, as well as the respiratory, circulatory, autonomic, and central neurological systems, as well as somatomotor activity and behavior patterns, were observed. Tremors, convulsions, salivation, diarrhea, lethargy, and sleep disturbances and coma should all be noted. Animals were fasted overnight and anesthetized at the end of the treatment, and blood was collected for hematologic and biochemical analyses. No mortality nor signs of toxicity during the treatment period were monitored. There was no substantial variation in body weight between the genders, and the animals showed no behavioral alterations. At necropsy, the liver and kidneys revealed no abnormalities. When compared to the control group, the hematological parameters RBC, HGB, MCV, CHCM, HCT, and PLT were significantly different for both genders. CRE levels in the blood were considerably lower in extract-treated females than in the control group. CHOL levels in males were found to be much lower.

For the sub-acute toxicity study, during the treatment phase, the animals were separated into four groups of ten (5 males and 5 females) and their body weights were recorded. Ethanol 51 percent (10 mL/kg) was given to the control group, whereas EEO was given once a day by oral gavage at dosages of 100, 200, and 400 mg/kg for 28 days. During the treatment period, the animals were examined for evidence of abnormalities. Animals were fasted overnight, anesthetized, and blood was collected for hematologic and biochemical analyses at the end of the treatment. Following euthanasia, liver and kidney samples were taken, fixed, and processed for histological analysis. In rats treated with varied doses of the extract for 28 days, no signs of toxicity or mortality were identified. The body weight of both genders followed a normal pattern, and necropsy revealed no abnormalities in the liver or kidneys. No behavioral changes were monitored over the course of the study either.

The liver and kidney histopathological findings of rats treated with 100, 200, and 400 mg/kg of the extract revealed normal morphological aspects. When compared to control animals, prolonged exposure to the olive leaf extract at different dosages (100, 200, and 400mg/kg) had no effect on any of the evaluated hematological parameters (RBC, HGB, HCT, MCV, MCHC, PLT, and WBC). Males exposed to EEO at dosages of 100 and 400 mg/kg had significantly higher blood BUN concentrations than the control group. Other metrics examined revealed no differences between the groups.

Overall, the ethanolic extract of Olea europaea L. leaves did not produce any toxicity in the experimental animal, and no mortality was observed for the doses supplied. Hematological, biochemical indicators and histopathology were normal, regardless of the gender or age of the animals. The olive leaf extract does not present toxicity when used in the same settings as this study.

Human Studies

i. Bonolive® studies

Sixteen women aged 18–75 (including pre- and post-menopausal groups) were given a single 250 mg serving of Bonolive® in a pharmacokinetic study without any reported adverse events.[25] Bonolive® was also given to 64 osteopenic individuals; they received either 250 mg per day of Bonolive® or placebo for 12 months (both groups also received 1000 mg of calcium per day).[75] The overall incidence of adverse events was similar between the two study groups. Two serious adverse events occurred (a right forearm fracture and a mammography result which raised suspicion of breast cancer but turned out to be incorrect); however, both events were in the placebo group. None of the adverse events were considered related to treatment. The most commonly occurring events were upper pulmonary tract infections (one from each group), mild dyspepsia (one from each group), mild

increase in systolic blood pressure (two in the treatment group, both in subjects with a history of hypertension), and back pain (two in the placebo group, both in subjects with a history of discopathy). In summary, no clinically relevant treatment-related adverse events were noted during the entire year-long study.

Bonolive's® effect in supporting the functionality and biomarkers of bone/cartilage metabolism and inflammation, in mid-aged people experiencing knee discomfort was assessed in a 6-month clinical study[76]. The study was a randomized, double-blind, experiment with two parallel groups in free-living healthy 124 mid-aged male and female individuals with moderate knee discomfort and loss of mobility.

The participants were randomized to one of two trial groups: (1) investigational substance, or (2) placebo. During the 6-month study, participants took one 125-mg Bonolive® or placebo capsule twice a day, at the start of the meal in the morning and evening. The investigational substance was 125 mg of Bonolive® per capsule, comprising 50 mg.Treatment with Bonolive® was well tolerated.

There were 114 adverse effects in total, 67 in the placebo group and 47 in the Bonolive® group. None of the adverse events were considered related to treatment. GI disorders (abdominal pain, nausea, dyspepsia, and musculoskeletal and connective tissue disorders were the most common adverse effects.

ii. Other olive leaf extract studies

With regard to other olive-leaf extracts, there were no treatment-related adverse events in a 30-week randomized, double-blinded, controlled cross-over study (with a six-week washout period) in 46 overweight male subjects aged 35–55 years that took four capsules per day of an olive leaf extract suspended in safflower oil.[11] The dose equated to daily consumption of 51.1 mg oleuropein and 9.7 mg hydroxytyrosol. Liver function tests revealed no differences between groups (parameters included AST, ALP, ALT and GGT). In addition, 500 mg per day of a hexane and ethanolic extract of olive leaves was given to subjects in a 14-week double-blind placebo-controlled study of 79 adults with type two diabetes, without reports of adverse events.[77]

Forty borderline hypertensive (untreated) monozygotic twins (age 18–60) were assigned to take 500 or 1000 mg per day of an olive leaf ethanolic extract (as oral tablets of EFLA®943 by Frutarom Switzerland Ltd., consisting of 18–26% oleuropein and 30–40% total polyphenols) for eight weeks, or were given advice on a favorable lifestyle.[78] The authors reported that no adverse events were observed throughout the trial. The same olive leaf extract (500 mg per day of EFLA®943) or Captopril (as the active-control) were randomly assigned to subjects with stage-1 hypertension for eight weeks in a double-blind, randomized, parallel study.[79] One hundred and sixty-two subjects completed the study. Safety endpoints included clinically significant changes in laboratory parameters such as those found in

hematology and clinical chemistry assessments. Slight shifts in some laboratory parameters were noted compared to baseline in several subjects from each group; however, they were not considered clinically relevant as they were all within normal ranges and they were very slight. The majority of adverse events in the study were considered mild (99.8%) and occurred similarly between groups. The most common events were coughing (4.5% in olive leaf extract and 7% in Captopril groups), and vertigo (5.9% in olive leaf extract and 6.3% in Captopril groups). One serious adverse event occurred in the olive leaf extract group; the subject suffered from severe anemia after persistent menorrhagia. The incident was considered related to the subject's history of abortion and curettage, and not related to consumption of the olive leaf extract. Coughing was considered likely related to Captopril, since it is an adverse event widely known to occur following intake of the drug. Mild events of vertigo, muscle discomfort and headaches were considered "possibly" related to both olive leaf extract and Captopril intake. All events had resolved by the end of the study.

A short abstract by Fonolla et al. describes a study in which 39 subjects were randomized into two groups; one group received 1,200 mg/day of an olive leaf extract called "Olivia®" and the other received placebo; both the test article and placebo capsules were given in divided doses (twice per day) for 28 days.[80] Plasma triglycerides, AST, ALT, creatinine and uric acid levels remained unchanged, while decreases in cholesterol levels occurred that were considered beneficial. No adverse events were mentioned.

Wong et al. conducted a 12-week randomized double-blind, placebo-controlled, cross-over trial on 37 adults 18–80 years old with BMI between 20 and 35 and baseline BP between 130–160 mmHg systolic and 85–100 mmHg diastolic with a combined formula of olive leaf extract, green coffee bean extract and beet powder.[81] Olive leaf extract (1000 mg, 160–240 mg oleuropein) accounted for more than 60% of the ingredients in the combined formulation. Four reported minor adverse events occurred during the 12-week intervention; vivid dreams, gastrointestinal discomfort, increased headache frequency and severity for a pre-existing migraine sufferer, and improved taste (n=1 for each); the first three occurred during the active treatment phase. One serious adverse event occurred in a participant who had been scheduled, prior to study enrollment, for a routine angiogram to determine stent size. The participant received the stent procedure without incident and completed the intervention within the study timeframe.

With regard to single dose administration, in a study of nine individuals who took capsules or liquid preparations of olive leaf extract containing up to 76.6 mg oleuropein and 14.5 hydroxytyrosol, no adverse effects were noted, and measured markers of liver function (AST, AST, ALP, GGT, and international normalized ratio) were unaltered.[11] Another single dose administration study of olive leaf extract was conducted on 18 individuals (9 males, 9 females), aged 19–40 years old who consumed a one-time dose of 1600 mg of olive leaf extract (400 mg per

capsule) delivering a total of 51.12 mg oleuropein and 9.67 mg hydroxytyrosol.[82] No adverse effects were noted, including measures taken to evaluate vascular function and inflammation levels.

6.1.4 Authoritative Safety Opinions

European Food Safety Authority (Health Claim Opinion)

While not directly related to a safety assessment, the European Food Safety Authority (EFSA) has stated a conclusion with regard to efficacy claims for olive polyphenols, which implies a certain degree of lack of concern for safety. When reviewing scientific substantiation for proposed health claims, EFSA's Panel on Dietetic Products, Nutrition and Allergies concluded that "a cause and effect relationship has been established between the consumption of olive oil polyphenols…and protection of LDL particles from oxidative damage."[83] The following health claim is thus allowable by EFSA:

- Consumption of olive oil polyphenols contributes to the protection of blood lipids from oxidative damage

In order to bear the claim, 5 mg of hydroxytyrosol and its derivatives (e.g., oleuropein complex and tyrosol) in olive oil should be consumed daily. The target population for the claim is considered the general population. The conditions of use specify 200 mg/day of polyphenols, 2–15 mg per day of hydroxytyrosyl or oleuropein complex, and 250–500 mg of an *Olea europaea* L. extract standardized to 4–23% oleuropein.

Note that while the claim uses the term "olive oil", EFSA's conclusion statement regarding the claim also mentions olive leaf: "The food constituent, polyphenols in olive (olive fruit, olive mill waste waters or olive oil, *Olea europaea* L. extract and leaf) standardized by their content of hydroxytyrosol and its derivatives (e.g., oleuropein complex), which is the subject of the health claims, is sufficiently characterized in relation to the claimed effects."

Novel Food Status

Olive leaf is listed in the European Commission's Novel Food catalogue as having "FS status", which is defined as follows: "According to information available to Member States competent authorities this product was used only as or in food supplements before 15 May 1997. Any other food uses of this product have to be authorized pursuant to the Novel Food Regulation."

Health Canada

The Health Canada natural health product monograph for orally administered olive leaf for adults was finalized in 2018.[84] It includes use as an antioxidant or diuretic,

in forms including dry, powder, tincture, or fluid extract (up to 3.5 g dried leaf per day), as a decoction (up to 5 g dried leaves or 10 g fresh leaves per single dose), or as an infusion (up to 8 g dried leaves per single dose, not to exceed 30 g dried leaves per day). It can also be used as an antioxidant as a standardized extract form (up to 500 mg per day and containing up to 20.8% oleuropein).

6.1.5 Allergenicity

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.[85] However, the olive leaves are harvested in the season when no pollen are produced. Moreover, the extraction process of Bonolive® makes the presence of pollen in the extract highly unlikely.

Contact (topical) allergy to olive oil is rare, and may result in eczema-type symptoms in sensitive subjects, although ingestion of the oil is often still tolerated.[86,87] Despite its common consumption, food allergy reactions to olive fruits is extremely rare, although it has been reported.[88]

We were unable to find any reports of allergic reactions to olive leaves, olive leaf extracts or oleuropein. On the contrary, there is some evidence to suggest that olive leaf polyphenols may be protective against allergic types of reactions (e.g., by inhibiting mast cell degranulation).[89] Bonolive® does not contain any of the allergens listed in Commission Directive 2007/68/EC.

6.1.6 History of Consumption

Humans

Polyphenol compounds from the olive tree have been consumed for millennia, especially in the Mediterranean region.[1] The so-called Mediterranean diet has been associated with many health benefits[3,4,90] considered largely due to its richness in olives and olive oils. Those who consume this diet have generally been reported to ingest up to 172 mg (68.5 ± 104.0 mg) of polyphenols from olives per day, including oleuropein, hydroxytyrosine, tyrosol, hydroxycinnamic acids, hydroxybenzoic acids, anthocyanidins, and more.[10,40]

The leaves of olive trees have also been consumed traditionally for health purposes; nineteenth century references cite olive leaf use as a febrifuge,[91,92] and various olive leaf extract products are currently sold in the marketplace as is shown in the section below entitled “Similar Products in the Marketplace”. Recently, interest in the high polyphenolic levels in olive leaves has led to the study of enhancing olive and other

edible oils with olive leaf extracts to increase phenolic concentrations which resist oxidative deterioration.[93,94]

Animals

Olive leaves have been used traditionally as animal feed in olive-producing regions, as the leaves are a major by-product of farming olive fruits. They have been studied as feed for animals including goats, sheep, rabbits, hens, pigs and cattle.[27,73,95-99] In their review of olive by-products for animal feed, Sansoucy et al. stated the following with regard to olive leaves: "ad libitum distribution to ruminants presents no special problems except that of the low nutritive value of the fodder" (additional supplements such as protein are recommended, as are also recommended for fodder use of straw or hay, and it was recommended that olive leaves be used fresh to increase their nutritive value).[95]

Supplementing hens' diets with 10 g/kg of olive leaves may protect the omega-3 fatty acids in the hens' eggs from deterioration.[96] Pigs supplemented with olive leaves (containing 2.2% oleuropein and 6.4% total polyphenols) at 25 g/kg in their diet showed improved quality of meat without adverse effects such as liver toxicity or compromising growth performance.[73] The pigs consumed approximately 2.5 kg of food per day, and weighed between 54 and 94 kg throughout the study. With a diet of 25 g/kg olive leaves (containing 2.2% oleuropein), consumption by the pigs was approximately 63 g of olive leaf or oleuropein per day, or 1.4 g of oleuropein per day, equivalent to 15–26 mg oleuropein/kg bw/day.

6.1.7 Past Sales and Reported Adverse Events

Since launching the ingredient in 2014, BioActor report that a total of circa 6000 kg of Bonolive® have been sold worldwide. Corresponding with more than 20 million daily doses. Over that time, no adverse events have been reported.

No FDA letters regarding concern for safety to companies that market products containing olive leaf extract were located. A search of MedWatch, FDA's adverse event reporting program, and FDA's Recalls, Market Withdrawals, & Safety Alerts search engine did not uncover any mention of olive leaf extract products.

There is one case report by Shaw (2016) of a possible adverse effect from use of an olive leaf extract that was located in the literature.[100] In this report, a 67-year-old woman suffered from severe hay fever and had tolerated 500 mg/day of olive leaf extract for two years with no adverse effects. She then began taking a dietary supplement containing olive leaf extract, horseradish root, and eyebright for sinus and hay fever relief after which her total olive leaf extract intake per day was equivalent to 5.5 g dry olive leaf/day (i.e., dry leaf equivalent). Her side effects included feeling more easily annoyed and argumentative and after several weeks of taking the recommended doses, she reported feeling tearful, angry, easily annoyed,

negative, reactive, and lacking control. Several days after discontinuing the sinus supplement, all of those traits disappeared. Shaw suggests that the hydroxytyrosol constituent of olive leaf extract may be responsible for these behavioral responses. The fact that she previously tolerated a different olive leaf extract supplement suggests that something else about the new supplement may have caused the effect. The dietary supplement was not reintroduced to see if the symptoms reappeared, which would have made for a stronger argument. There is also a lack of explanation of the possible role of constituents in the horseradish and eyebright in contributing to the patient's mood changes.

6.1.8 Similar Products in the Marketplace

A general Internet search as well as searches of several large distributors of dietary supplements resulted in numerous findings of olive leaf extract products, illustrating that ingredients relatively similar to Bonolive® are widely available in the U.S. Despite this prevalence, we are unaware of any adverse events attributed to olive leaf extracts. Some examples are listed in Table 15 below.

Table 15. U.S. Products Containing Olive Leaf Extracts[101]

Company	Product Name	Serving Size
Barlean's	Olive Leaf Complex Softgels	225 mg olive leaf extract (minimum 40% oleuropein) 90 mg oleuropein
BulkSupplements.com	Olive Leaf Extract (20% Oleuropein)	750 mg olive leaf extract (20% oleuropein)
Douglas Laboratories	Olive Leaf Extract	500 mg olive leaf extract (20% oleuropein)
Gaia Herbs	Olive Leaf	900 mg olive leaf extract
Hardy Nutritionals	Olive Leaf Extract	500 mg olive leaf extract (17% oleuropein)
Natural Factors	Olive Leaf	500 mg olive leaf extract (minimum of 75 mg oleuropein)
Nature's Sunshine	Olive Leaf Extract	420 mg olive leaf extract (12% oleuropein)
NOW	Olive Leaf Extract 500 mg	500 mg olive leaf extract (minimum 6% oleuropein)
Nutrients for Health	Olive Leaf Extract	500 mg olive leaf extract
Pure	Olive Leaf	940 mg olive leaf extract (188mg oleuropein)
Roex	Oleuropein	500 mg olive leaf extract (20% oleuropein)
Seeking Health	Olive Leaf Extract 250 mg	250 mg olive leaf extract (20% oleuropein)
Solaray	Olive Leaf Extract 250 mg	250 mg olive leaf extract (minimum 22% oleuropein)
Triquetra Health	Total Olive	400mg olive leaf extract (minimum 40% oleuropein) 160 mg oleuropein

Nature's Plus	Olive Leaf—extended release	500 mg olive leaf extract, (minimum 6% oleuropein)
Nature's Way	Olive Leaf –Standardized	250 mg olive leaf extract (20% oleuropein 180 mg olive leaf
Now Foods	Olive Leaf Extract	500 mg olive leaf extract (minimum of 6% oleuropein)
Only Natural	Olive Leaf Extract	500 mg olive leaf extract minimum of 6% oleuropeins)
Paradise Herbs	Olive Leaf	250 mg olive leaf extract (minimum 15% oleuropein)
Solaray	Olive Leaf	1000 mg olive leaf extract (minimum of 170 mg oleuropein)
Vitacost	Olive Leaf Extract	500 mg olive leaf extract (minimum of 18% oleuropein)
VitaminsDirect	VitaminsDirect Olive Leaf Extract	500 mg olive leaf extract

6.1.9 Current Regulatory Status

A thorough search for the current regulatory status of olive leaf extract, relevant to its use in food in the United States, was conducted. Searched entities included: *Olea europaea*, Olive leaf extract, Olive leaf, Oleaceae, Olea, Olive, Oleuropein. No specific findings with regard to olive leaf extract were found.

With regard to olive-related products, four FDA GRAS notices (GRN No. 459, GRN No. 600, GRN No. 726, and GRN No. 978) were found in the FDA GRAS Notice Inventory database.

GRN No. 459 is for an olive pulp extract; the notification was filed in 2013 by Phenofarm (Rome, Italy). At the notifier's request, FDA ceased to evaluate the notice (the reason for this is unknown).

GRN No. 600 is for almost pure hydroxytyrosol (>99% pure), a synthetic polyphenol (naturally found in olives and olive leaves). Seprox Biotech S.L. (Spain) filed the notification in September of 2015. The intended use for hydroxytyrosol is as an antioxidant and antimicrobial agent in conventional foods such as non-alcoholic beverages, fats and oils, fresh and processed fruits/vegetables and juices and gravy and sauces at levels of 5.0 mg per serving. It is not intended for use in foods intended for infants and children. The GRAS determination was based on scientific procedures including research on olive oil, table olives and olive extracts enriched with hydroxytyrosol. The hydroxytyrosol is not intended for use in meat or poultry. FDA gave notice not to have any further questions on May 13, 2016.

GRN No. 726 is for a polyphenol preparation from olive fruits containing >40% hydroxytyrosol. DSM filed the notification in August 2017. FDA gave notice not to have further questions on February 28, 2018. DSM's product is proposed for use in 11 broad food categories: bakery products; beverages; dairy products and substitutes; desserts; fats and oils; fruit juices and nectars; dry seasoning mixes for

meat, poultry, and fish; chewing gum; sauces, dips, gravies, and condiments; snacks; and vegetable juices to deliver 5 to10 mg of hydroxytyrosol per serving of food.

Finally, GRN No. 978 refers to an aqueous olive pulp extract containing >3.5% hydroxytyrosol. Oliphenol LLC. filed the notification in October 2020. FDA gave notice not to have further questions on December 10, 2021. The extract is intended to be used as an ingredient, but not intended for use in infant formula, meat, poultry, non-exempt egg products, catfish, or any products that would require additional regulatory review by USDA.

Additionally, an NDI notification was submitted to FDA in 2006 by Seppic, Inc. (Fairfield, NJ) for a product called Polivols (an olive fruit extract). However, FDA did not believe the ingredient was described/characterized well enough to determine its relationship to other olive products, and hence its safety.

6.2 Data and Information Appearing Inconsistent with the GRAS Conclusion

In a study to evaluate the effect of repeated dose intake of an olive leaf extract (called "D-lenolate", not otherwise characterized) on the livers of mice, female ICR mice were divided into four groups of ten, and were given an olive leaf extract as a percentage of the diet for 14 weeks.[102] The concentrations in the diet were: 0%, 0.25%, 0.5% and 0.75% for groups 0, 1, 2 and 3, respectively.

In the study, the mortality rates were 10, 0, 20 and 50% for groups 0, 1, 2 and 3, respectively. No changes in behavior were noted. Some animals in groups 2 and 3 exhibited icterus, including 20% in group 2 and 90% in group 3. There was a significant difference between final body weights between group 0 (36.86 g) and group 3 (27.22 g), $p = 0.012$, while food intake was not different between test article groups.

Relative liver weights were similar for groups 2 and 3 but were higher than those from groups 0 and 1. Macroscopic changes occurred in the livers of all groups that consumed the test article, and included greenish liver staining, bile duct dilatation and gall bladder distension (control livers were normal). No macroscopic changes were noted in the heart, kidneys, bladder, spleen, or lungs in any of the groups.

Serum enzyme activities of ALT and ALP increased significantly in groups 2 and 3. Total bilirubin increased in groups 2 and 3, although the increase was not statistically significantly different. Histopathologically, liver architecture alternations and hepatic fibrosis were observed in groups 2 and 3 and were more severe in group 3. All groups exposed to the extract presented bile duct hyperplasia, cholestasis, hepatocyte necrosis and inflammatory infiltrate with the severity of injuries increasing in line with increases in consumption. Liver mitosis was present in test article groups, with the highest levels in group 3. Groups 2 and 3 also had

increased reticulin expression in the liver parenchyma and portal space, as well as increased collagen expression. Histopathological changes were not identified in any other organs.

Unfortunately, the authors didn't calculate (or provide enough data for readers to calculate) the amount of the extract that was consumed by the mice in the different dose groups. A very general estimate was attempted based on a typical 4–5 g/day diet of mice and 18–40 g weight of adult females.[58] Such estimations suggest that animals in the 0.5% group may have received approximately 500–1111 mg/kg bw/day and animals in the 0.75% group may have received approximately 625–1389 mg/kg bw/day during the study (or using Lehman's conversion factor for mice, approximately 750 and 1125 mg/kg bw/day for the 0.5% and 0.75% groups, respectively). The other key piece of information that is missing from this study is characterization of the test article, thus it is impossible to compare it to Bonolive®. The study was also not OECD compliant. The fact that no liver findings were seen in the rats during the 14- and 90-day repeated dose studies on Bonolive® seems to suggest that the test article used in this mouse study may have been significantly different than Bonolive®, the doses may have been significantly different, or there are differences between effects in rats versus this particular strain of mice under these testing conditions (it is also possible the mice received much higher doses of the test article than our rough calculations suggest). Interestingly, in the study by Kumral and colleagues discussed above,[64] an olive leaf extract led to decreases in ALT (and AST) in rats given doxorubicin, which appears in contrast to this study.

In another non-OECD compliant study, a safety evaluation on daily ingestion of free and total polyphenolic compounds from fruits and leaves of a particular cultivar of olive tree (Picual) were studied for seven weeks in rats.[103] One mL of a water/tween solution containing 400, 800, 1200 or 1600 ppm of phenolics, or 200 ppm butylated hydroxyl toluene (BHT)) was given to rats via gavage daily, and several parameters were measured to assess safety. Both BHT and 1600 ppm phenolics consumption resulted in significant increases in serum AST and ALT values. The 1600 ppm solution caused a slight increase in kidney and liver weights, while BHT caused significant enlargement of these organs. BHT and 1600 ppm also led to histopathological changes in the kidney and liver tissues, while at 1200 ppm tissues didn't differ from those of the controls. The ppm doses were not translated to mg/kg by the authors, and the test article was not specifically characterized, thus it is again difficult to compare these results to those of other studies with regard to doses and/or constituents that may lead to these types of findings.

A bacterial reverse mutation and chromosomal aberration assay using Chinese Hamster ovary cells revealed evidence of mutagenic activity of an olive pulp extract containing 6% olive polyphenols (HIDROX™, CreAgri, Inc. California) at high doses with S9 metabolic activation.[65] In the bacterial reverse mutation study, evidence of mutagenic activity was detected in the plate incorporation test at concentrations of 1000 and 2500 µg/plate of the extract (but not at the high dose of

5000 µg/plate) with *S. typhimurium* tester strains TA98 and TA100, but not in tester strains TA97a, TA1535 or *E. coli* strain WP2 uvr A. These findings were confirmed in the more sensitive preincubation test (at doses of 1000 and 2500 µg/plate), but only with metabolic activation. The authors stated that inconsistencies between the regular and repeat trials, the antibacterial properties of the extract and observations of positive findings at only certain dose groups complicate the interpretation of the findings. In the chromosomal aberration study, a significant increase in the percentage of aberrant cells was noted at the highest concentration (1000 µg/mL) with metabolic activation. Yet in vivo rat micronucleus evaluations performed using gavage doses of the extract showed negative findings. After single doses of 1000, 1500 or 2000 mg/kg bw/day, the number of micronucleated PCEs were not significantly increased in any test article group. Repeated doses were given for 28 days, and preliminary scanning of slides showed a negative response at 2000 mg/kg bw/day, thus scoring proceeded on day 29 with the high dose (5000 mg/kg bw/day) group, in conjunction with the positive control and negative controls. Numbers of micronucleated PCEs were not increased in males or females as compared to the negative control. Thus, the extract was not considered mutagenic in the in vivo assay. Importantly, as discussed above, Bonolive® showed no evidence of mutagenicity in a bacterial reverse mutation test and in an vitro mammalian chromosomal aberration test, nor was any genotoxic activity observed in an in vivo mouse micronucleus test at concentrations up to the limit dose of 2000 mg/kg bw/d.[54]

We are not aware of any other data and/or information that are, or may appear to be, inconsistent with our conclusion of GRAS status.

6.3 Information that is Exempt from Disclosure under FOIA

There is no data or information in this GRAS notice that is considered exempt from disclosure under the U.S. Freedom of Information Act (FOIA).

6.4 Basis for the GRAS Conclusion

The scientific procedures forming the data of the safety assessment comprise the technical element of the GRAS standard. The common knowledge element is comprised of the general availability of the pivotal data establishing the technical element. Together, the technical element and the common knowledge element form the basis for the GRAS conclusion of Bonolive®.

6.4.1 Technical Element

Bonolive®, a water-soluble extract of olive leaves, has been the subject of a thorough safety assessment described above. The safety of this ingredient is supported by toxicological studies in animals, clinical studies in humans without occurrence of

serious adverse events, and the history of olive leaf consumption by humans and animals.

The totality of evidence for the safety of Bonolive® includes a ninety-day repeated-dose oral toxicity study on Bonolive® in rats, in which the NOAEL was 1000 mg/kg bw/day in male and female Wistar rats, the highest dose level tested. A bacterial reverse mutation test, an in vitro mammalian chromosomal aberration test, and an in vivo mammalian micronucleus test establish the lack of genotoxic potential of Bonolive®. There has been a lack of adverse events reported in published clinical trials using various olive leaf extracts (including several trials with Bonolive®), and over 6000 kg of Bonolive® have been sold worldwide for consumption thus far without reported adverse events following ingestion of over 20 million doses. There is a long history of human consumption of olive polyphenol products in general and a history of safe use of olive leaf as a feed for animals. The totality of safety evidence also includes EFSA's decision to allow a health claim for olive polyphenols (including those from olive leaf). Lastly, the high-quality control standards for this ingredient, as described in the Manufacturing, Production and Quality Management section of this dossier adds to the totality of safety data.

The intended use of Bonolive® is as an ingredient in a number of food categories at concentrations reported in Tables 1 and 7. As discussed above, due to its extremely bitter taste, it is very likely that Bonolive® will often be utilized at levels significantly below the maximum concentrations stated in the tables. The maximum estimated lifetime daily exposure to Bonolive® based on its intended uses relative to body weight by the 90th percentile consumer, as calculated using Creme software with a 20% presence probability factor, was by males aged 2–11 years, at 9.9 mg/kg bw/day, equivalent to up to 5.4 mg/kg bw/day oleuropein. The 90th percentile estimated exposure to Bonolive® for the total population (ages 2 years and above) was 6.5 mg/kg bw/day (equivalent to a maximum of approximately 3.6 mg/kg bw/day oleuropein). As discussed previously, oleuropein consumption from other sources in the diet is considered essentially negligible compared to the Bonolive® intake estimates, due to the fairly low level of oleuropein found in olive oil (the main intake source).

Using the results of the 90-day repeated dose study on Bonolive® in rats, a margin of safety can be calculated by dividing the NOAEL by the estimated daily intake for each population. The resulting margins of safety are approximately 101 for males aged 2–11 years (1000/9.9), and approximately 154 for the total population (1000/6.5).

These safety margins are considered reasonable for this ingredient based on the totality of safety evidence. The exposure estimates are still considered likely over-estimates of what true consumption will be. For example, assuming a US population of 213,300,000 individuals over the age of 25, an exposure for adults of approximately 500 mg/day would lead to an annual intake of nearly 39,000,000 kg

of Bonolive® in the US, which is several orders of magnitude higher than BioActor's highest sales estimates. The extremely bitter taste and cost of the branded Bonolive® ingredient will work to self-limit its use to some degree and are expected to lead to addition levels lower than the maximums stated in Tables 1 and 7.

Additionally, the NOAEL of the 90-day study was the limit dose and the highest dose tested, which suggests that if higher levels were to be tested in a similar toxicological study, the NOAEL would most likely be higher, increasing confidence that the ingredient is safe for consumption. The fact that olive leaves have been utilized as animal feed in many different species without adverse effects (at levels, for example, of 10 g/kg in hen feed[96] and 25 mg/kg in pig feed, equivalent to approximately 15–26 mg/kg bw/day oleuropein in pigs[73]) also supports that the current estimated human exposure levels would not be of safety concern. Finally, the general consensus that olive polyphenols have various health benefits, and the numerous olive leaf extract products on the market without serious adverse effects reported corroborates the safety.

Overall, the totality of evidence supports a conclusion that the intended use of Bonolive® is reasonably certain to be safe when ingested by humans under the conditions of its intended use.

6.4.2 Common Knowledge Element

The scientific studies, performed in laboratory animals and humans and herein reported that provide the basis of this GRAS determination by scientific procedures are published and available in the public domain. Part 7 of this notification contains the citations for the published studies. This published data fulfils the requirement for general availability of the pivotal scientific data contributing to the technical element of the GRAS standard and provides reasonable certainty that consumption of Bonolive® for its intended use is not harmful. The general availability of the pivotal safety data discussed herein satisfies the common knowledge element of this GRAS conclusion.

Part 7: Supporting Data and Information

7.1 Data and Information that are *not* Generally Available

All of the information described in this GRAS notice is generally available.

7.2 References that *are* Generally Available

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7.3 Appendix A. Full List of NHANES Food Codes Used for Exposure Estimates

Group Code	Group Name	Food Code	Food Name
114	Yogurt	11446000	Fruit and low-fat yogurt parfait
114	Yogurt	11427000	Yogurt, chocolate, non-fat milk
114	Yogurt	11425000	Yogurt, chocolate, NS as to type of milk
114	Yogurt	11426000	Yogurt, chocolate, whole milk
114	Yogurt	11460160	Yogurt, frozen, chocolate, low-fat milk
114	Yogurt	11460200	Yogurt, frozen, chocolate, non-fat milk
114	Yogurt	11460400	Yogurt, frozen, chocolate, non-fat milk, with low-calorie sweetener
114	Yogurt	11460100	Yogurt, frozen, chocolate, NS as to type of milk
114	Yogurt	11460430	Yogurt, frozen, chocolate, whole milk
114	Yogurt	11461000	Yogurt, frozen, chocolate-coated
114	Yogurt	11461250	Yogurt, frozen, cone, chocolate
114	Yogurt	11461280	Yogurt, frozen, cone, chocolate, low-fat milk
114	Yogurt	11461260	Yogurt, frozen, cone, flavors other than chocolate
114	Yogurt	11461270	Yogurt, frozen, cone, flavors other than chocolate, low-fat milk
114	Yogurt	11460170	Yogurt, frozen, flavors other than chocolate, low-fat milk
114	Yogurt	11460300	Yogurt, frozen, flavors other than chocolate, nonfat milk
114	Yogurt	11460410	Yogurt, frozen, flavors other than chocolate, nonfat milk, with low-calorie sweetener
114	Yogurt	11460000	Yogurt, frozen, flavors other than chocolate, NS as to type of milk
114	Yogurt	11460440	Yogurt, frozen, flavors other than chocolate, whole milk
114	Yogurt	11460250	Yogurt, frozen, flavors other than chocolate, with sorbet or sorbet-coated
114	Yogurt	11460150	Yogurt, frozen, NS as to flavor, low-fat milk
114	Yogurt	11460190	Yogurt, frozen, NS as to flavor, nonfat milk
114	Yogurt	11459990	Yogurt, frozen, NS as to flavor, NS as to type of milk
114	Yogurt	11460420	Yogurt, frozen, NS as to flavor, whole milk
114	Yogurt	11461200	Yogurt, frozen, sandwich
114	Yogurt	11432000	Yogurt, fruit, low fat milk
114	Yogurt	11432500	Yogurt, fruit, low fat milk, light
114	Yogurt	11433000	Yogurt, fruit, nonfat milk
114	Yogurt	11433500	Yogurt, fruit, nonfat milk, light
114	Yogurt	11430000	Yogurt, fruit, NS as to type of milk
114	Yogurt	11431000	Yogurt, fruit, whole milk
114	Yogurt	11428000	Yogurt, Greek, chocolate, nonfat
114	Yogurt	11434010	Yogurt, Greek, fruit, low fat
114	Yogurt	11434020	Yogurt, Greek, fruit, nonfat

114	Yogurt	11434000	Yogurt, Greek, fruit, whole milk
114	Yogurt	11411410	Yogurt, Greek, plain, low fat
114	Yogurt	11411420	Yogurt, Greek, plain, nonfat milk
114	Yogurt	11411400	Yogurt, Greek, plain, whole milk
114	Yogurt	11424510	Yogurt, Greek, vanilla, low fat
114	Yogurt	11424520	Yogurt, Greek, vanilla, nonfat
114	Yogurt	11424500	Yogurt, Greek, vanilla, whole milk
114	Yogurt	11410000	Yogurt, NS as to type of milk or flavor
114	Yogurt	11411200	Yogurt, plain, low fat milk
114	Yogurt	11411300	Yogurt, plain, nonfat milk
114	Yogurt	11411010	Yogurt, plain, NS as to type of milk
114	Yogurt	11411100	Yogurt, plain, whole milk
114	Yogurt	11422000	Yogurt, vanilla, low fat milk
114	Yogurt	11422100	Yogurt, vanilla, low fat milk, light
114	Yogurt	11423000	Yogurt, vanilla, nonfat milk
114	Yogurt	11424000	Yogurt, vanilla, nonfat milk, light
114	Yogurt	11420000	Yogurt, vanilla, NS as to type of milk
114	Yogurt	11421000	Yogurt, vanilla, whole milk
115	Flavored milk and milk drinks, fluid	11513300	Chocolate milk, made from dry mix with fat free milk (skim)
115	Flavored milk and milk drinks, fluid	11513200	Chocolate milk, made from dry mix with low fat milk (1%)
115	Flavored milk and milk drinks, fluid	11513310	Chocolate milk, made from dry mix with non-dairy milk
115	Flavored milk and milk drinks, fluid	11513150	Chocolate milk, made from dry mix with reduced fat milk (2%)
115	Flavored milk and milk drinks, fluid	11513100	Chocolate milk, made from dry mix with whole milk
115	Flavored milk and milk drinks, fluid	11513000	Chocolate milk, made from dry mix, NS as to type of milk
115	Flavored milk and milk drinks, fluid	11513804	Chocolate milk, made from light syrup with fat free milk (skim)
115	Flavored milk and milk drinks, fluid	11513803	Chocolate milk, made from light syrup with low fat milk (1%)
115	Flavored milk and milk drinks, fluid	11513805	Chocolate milk, made from light syrup with non-dairy milk
115	Flavored milk and milk drinks, fluid	11513802	Chocolate milk, made from light syrup with reduced fat milk (2%)
115	Flavored milk and milk drinks, fluid	11513801	Chocolate milk, made from light syrup with whole milk
115	Flavored milk and milk drinks, fluid	11513800	Chocolate milk, made from light syrup, NS as to type of milk
115	Flavored milk and milk drinks, fluid	11513370	Chocolate milk, made from reduced sugar mix with fat free milk (skim)
115	Flavored milk and milk drinks, fluid	11513365	Chocolate milk, made from reduced sugar mix with low fat milk (1%)
115	Flavored milk and milk drinks, fluid	11513375	Chocolate milk, made from reduced sugar mix with non-dairy milk
115	Flavored milk and milk drinks, fluid	11513360	Chocolate milk, made from reduced sugar mix with reduced fat milk (2%)
115	Flavored milk and milk drinks, fluid	11513355	Chocolate milk, made from reduced sugar mix with whole milk

115	Flavored milk and milk drinks, fluid	11513350	Chocolate milk, made from reduced sugar mix, NS as to type of milk
115	Flavored milk and milk drinks, fluid	11513854	Chocolate milk, made from sugar free syrup with fat free milk (skim)
115	Flavored milk and milk drinks, fluid	11513853	Chocolate milk, made from sugar free syrup with low fat milk (1%)
115	Flavored milk and milk drinks, fluid	11513855	Chocolate milk, made from sugar free syrup with non-dairy milk
115	Flavored milk and milk drinks, fluid	11513852	Chocolate milk, made from sugar free syrup with reduced fat milk (2%)
115	Flavored milk and milk drinks, fluid	11513851	Chocolate milk, made from sugar free syrup with whole milk
115	Flavored milk and milk drinks, fluid	11513850	Chocolate milk, made from sugar free syrup, NS as to type of milk
115	Flavored milk and milk drinks, fluid	11513700	Chocolate milk, made from syrup with fat free milk (skim)
115	Flavored milk and milk drinks, fluid	11513600	Chocolate milk, made from syrup with low fat milk (1%)
115	Flavored milk and milk drinks, fluid	11513750	Chocolate milk, made from syrup with non-dairy milk
115	Flavored milk and milk drinks, fluid	11513550	Chocolate milk, made from syrup with reduced fat milk (2%)
115	Flavored milk and milk drinks, fluid	11513500	Chocolate milk, made from syrup with whole milk
115	Flavored milk and milk drinks, fluid	11513400	Chocolate milk, made from syrup, NS as to type of milk
115	Flavored milk and milk drinks, fluid	11511000	Chocolate milk, NFS
115	Flavored milk and milk drinks, fluid	11511300	Chocolate milk, ready to drink, fat free (skim)
115	Flavored milk and milk drinks, fluid	11511400	Chocolate milk, ready to drink, low fat (1%)
115	Flavored milk and milk drinks, fluid	11511200	Chocolate milk, ready to drink, reduced fat (2%)
115	Flavored milk and milk drinks, fluid	11511550	Chocolate milk, ready to drink, reduced sugar, NS as to milk
115	Flavored milk and milk drinks, fluid	11511100	Chocolate milk, ready to drink, whole
115	Flavored milk and milk drinks, fluid	11531500	Eggnog, low-fat / light
115	Flavored milk and milk drinks, fluid	11531000	Eggnog, regular
115	Flavored milk and milk drinks, fluid	11553130	Fruit smoothie juice drink, with dairy
115	Flavored milk and milk drinks, fluid	11553100	Fruit smoothie, NFS
115	Flavored milk and milk drinks, fluid	11553110	Fruit smoothie, with whole fruit and dairy
115	Flavored milk and milk drinks, fluid	11553120	Fruit smoothie, with whole fruit and dairy, added protein
115	Flavored milk and milk drinks, fluid	11514140	Hot chocolate / Cocoa, made with dry mix and fat free milk (skim)
115	Flavored milk and milk drinks, fluid	11514130	Hot chocolate / Cocoa, made with dry mix and low-fat milk (1%)
115	Flavored milk and milk drinks, fluid	11514150	Hot chocolate / Cocoa, made with dry mix and non-dairy milk

115	Flavored milk and milk drinks, fluid	11514120	Hot chocolate / Cocoa, made with dry mix and reduced fat milk (2%)
115	Flavored milk and milk drinks, fluid	11514100	Hot chocolate / Cocoa, made with dry mix and water
115	Flavored milk and milk drinks, fluid	11514110	Hot chocolate / Cocoa, made with dry mix and whole milk
115	Flavored milk and milk drinks, fluid	11514350	Hot chocolate / Cocoa, made with no sugar added dry mix and fat free milk (skim)
115	Flavored milk and milk drinks, fluid	11514340	Hot chocolate / Cocoa, made with no sugar added dry mix and low-fat milk (1%)
115	Flavored milk and milk drinks, fluid	11514360	Hot chocolate / Cocoa, made with no sugar added dry mix and non-dairy milk
115	Flavored milk and milk drinks, fluid	11514330	Hot chocolate / Cocoa, made with no sugar added dry mix and reduced fat milk (2%)
115	Flavored milk and milk drinks, fluid	11514310	Hot chocolate / Cocoa, made with no sugar added dry mix and water
115	Flavored milk and milk drinks, fluid	11514320	Hot chocolate / Cocoa, made with no sugar added dry mix and whole milk
115	Flavored milk and milk drinks, fluid	11512010	Hot chocolate / Cocoa, ready to drink
115	Flavored milk and milk drinks, fluid	11512030	Hot chocolate / Cocoa, ready to drink, made with non-dairy milk
115	Flavored milk and milk drinks, fluid	11512120	Hot chocolate / Cocoa, ready to drink, made with non-dairy milk and whipped cream
115	Flavored milk and milk drinks, fluid	11512020	Hot chocolate / Cocoa, ready to drink, made with nonfat milk
115	Flavored milk and milk drinks, fluid	11512110	Hot chocolate / Cocoa, ready to drink, made with nonfat milk and whipped cream
115	Flavored milk and milk drinks, fluid	11512100	Hot chocolate / Cocoa, ready to drink, with whipped cream
115	Flavored milk and milk drinks, fluid	11551050	Licuado / Batido (milk fruit drink)
115	Flavored milk and milk drinks, fluid	11541400	Milk shake with malt
115	Flavored milk and milk drinks, fluid	11543000	Milk shake, bottled, chocolate
115	Flavored milk and milk drinks, fluid	11543010	Milk shake, bottled, flavors other than chocolate
115	Flavored milk and milk drinks, fluid	11542100	Milk shake, fast food, chocolate
115	Flavored milk and milk drinks, fluid	11542200	Milk shake, fast food, flavors other than chocolate
115	Flavored milk and milk drinks, fluid	11541110	Milk shake, home recipe, chocolate
115	Flavored milk and milk drinks, fluid	11541130	Milk shake, home recipe, chocolate, light
115	Flavored milk and milk drinks, fluid	11541120	Milk shake, home recipe, flavors other than chocolate
115	Flavored milk and milk drinks, fluid	11541135	Milk shake, home recipe, flavors other than chocolate, light
115	Flavored milk and milk drinks, fluid	11526000	Milk, malted, chocolate, made with milk
115	Flavored milk and milk drinks, fluid	11525000	Milk, malted, natural flavor, made with milk
115	Flavored milk and milk drinks, fluid	11513384	Nesquik, chocolate milk, made from dry mix with fat free milk (skim)

115	Flavored milk and milk drinks, fluid	11513383	Nesquik, chocolate milk, made from dry mix with low fat milk (1%)
115	Flavored milk and milk drinks, fluid	11513385	Nesquik, chocolate milk, made from dry mix with non-dairy milk
115	Flavored milk and milk drinks, fluid	11513382	Nesquik, chocolate milk, made from dry mix with reduced fat milk (2%)
115	Flavored milk and milk drinks, fluid	11513381	Nesquik, chocolate milk, made from dry mix with whole milk
115	Flavored milk and milk drinks, fluid	11513380	Nesquik, chocolate milk, made from dry mix, NS as to type of milk
115	Flavored milk and milk drinks, fluid	11513394	Nesquik, chocolate milk, made from no sugar added dry mix with fat free milk (skim)
115	Flavored milk and milk drinks, fluid	11513393	Nesquik, chocolate milk, made from no sugar added dry mix with low fat milk (1%)
115	Flavored milk and milk drinks, fluid	11513395	Nesquik, chocolate milk, made from no sugar added dry mix with non-dairy milk
115	Flavored milk and milk drinks, fluid	11513392	Nesquik, chocolate milk, made from no sugar added dry mix with reduced fat milk (2%)
115	Flavored milk and milk drinks, fluid	11513391	Nesquik, chocolate milk, made from no sugar added dry mix with whole milk
115	Flavored milk and milk drinks, fluid	11513390	Nesquik, chocolate milk, made from no sugar added dry mix, NS as to type of milk
115	Flavored milk and milk drinks, fluid	11511610	Nesquik, chocolate milk, ready to drink, fat free (skim)
115	Flavored milk and milk drinks, fluid	11511600	Nesquik, chocolate milk, ready to drink, low fat (1%)
115	Flavored milk and milk drinks, fluid	11511700	Nesquik, chocolate milk, ready to drink, low fat (1%), no sugar added
115	Flavored milk and milk drinks, fluid	11519205	Strawberry milk, fat free (skim)
115	Flavored milk and milk drinks, fluid	11519200	Strawberry milk, low fat (1%)
115	Flavored milk and milk drinks, fluid	11519040	Strawberry milk, NFS
115	Flavored milk and milk drinks, fluid	11519215	Strawberry milk, non-dairy
115	Flavored milk and milk drinks, fluid	11519105	Strawberry milk, reduced fat (2%)
115	Flavored milk and milk drinks, fluid	11519050	Strawberry milk, whole
115	Flavored milk and milk drinks, fluid	11560000	Yoo-hoo, chocolate milk drink
118	Milk, dry, and powdered mixtures with dry milk, not reconstituted	11830160	Chocolate beverage powder, dry mix, not reconstituted
118	Milk, dry, and powdered mixtures with dry milk, not reconstituted	11830165	Chocolate beverage powder, reduced sugar, dry mix, not reconstituted
118	Milk, dry, and powdered mixtures with dry milk, not reconstituted	11830150	Cocoa powder, not reconstituted (no dry milk)
118	Milk, dry, and powdered mixtures with dry milk, not reconstituted	11830115	Hot chocolate / Cocoa, dry mix, no sugar added, not reconstituted

118	Milk, dry, and powdered mixtures with dry milk, not reconstituted	11830100	Hot chocolate / Cocoa, dry mix, not reconstituted
118	Milk, dry, and powdered mixtures with dry milk, not reconstituted	11813000	Milk, dry, not reconstituted, fat free (skim)
118	Milk, dry, and powdered mixtures with dry milk, not reconstituted	11812000	Milk, dry, not reconstituted, low fat (1%)
118	Milk, dry, and powdered mixtures with dry milk, not reconstituted	11810000	Milk, dry, not reconstituted, NS as to fat content
118	Milk, dry, and powdered mixtures with dry milk, not reconstituted	11811000	Milk, dry, not reconstituted, whole
118	Milk, dry, and powdered mixtures with dry milk, not reconstituted	11830260	Milk, malted, dry mix, not reconstituted
118	Milk, dry, and powdered mixtures with dry milk, not reconstituted	11830400	Strawberry beverage powder, dry mix, not reconstituted
118	Milk, dry, and powdered mixtures with dry milk, not reconstituted	11825000	Whey, sweet, dry
424	Coconut beverages	42402010	Coconut cream (liquid expressed from grated coconut meat), canned, sweetened
424	Coconut beverages	42401010	Coconut milk, used in cooking (liquid expressed from grated coconut meat, water added)
424	Coconut beverages	42404010	Coconut water, sweetened
424	Coconut beverages	42403010	Coconut water, unsweetened (liquid from coconuts)
532	Cookies	53201000	Cookie, NFS
532	Cookies	53202000	Cookie, almond
532	Cookies	53205260	Cookie, bar, with chocolate
532	Cookies	53206030	Cookie, chocolate chip, reduced fat
532	Cookies	53206500	Cookie, chocolate, made with rice cereal
532	Cookies	53206550	Cookie, chocolate, made with oatmeal and coconut (no-bake)
532	Cookies	53207020	Cookie, chocolate or fudge, reduced fat
532	Cookies	53207050	Cookie, chocolate, with chocolate filling or coating, fat free
532	Cookies	53211000	Cookie bar, with chocolate, nuts, and graham crackers
532	Cookies	53220000	Cookie, fruit-filled bar
532	Cookies	53220010	Cookie, fruit-filled bar, fat free
532	Cookies	53220030	Cookie, fig bar
532	Cookies	53220040	Cookie, fig bar, fat free
532	Cookies	53223100	Cookie, granola
532	Cookies	53231400	Cookie, multigrain, high fiber
532	Cookies	53233000	Cookie, oatmeal
532	Cookies	53233010	Cookie, oatmeal, with raisins
532	Cookies	53233040	Cookie, oatmeal, reduced fat, NS as to raisins

532	Cookies	53235600	Cookie, Pfeffernusse
532	Cookies	53236100	Cookie, pumpkin
532	Cookies	53237000	Cookie, raisin
532	Cookies	53239010	Cookie, shortbread, reduced fat
532	Cookies	53241510	Marie biscuit
532	Cookies	53241600	Cookie, butter, or sugar, with fruit and/or nuts
532	Cookies	53246000	Cookie, tea, Japanese
532	Cookies	53247050	Cookie, vanilla wafer, reduced fat
532	Cookies	53260030	Cookie, chocolate chip, sugar free
532	Cookies	53260200	Cookie, oatmeal, sugar free
532	Cookies	53260400	Cookie, sugar or plain, sugar free
532	Cookies	53260500	Cookie, sugar wafer, sugar free
537	Bars	53714520	Breakfast bar, cereal crust with fruit filling, low-fat
537	Bars	53714510	Breakfast bar, date, with yogurt coating
537	Bars	53714500	Breakfast bar, NFS
537	Bars	53710400	Fiber One Chewy Bar
537	Bars	53714220	Granola bar with nuts, chocolate-coated
537	Bars	53714200	Granola bar, chocolate coated, NFS
537	Bars	53714250	Granola bar, coated with non-chocolate coating
537	Bars	53714300	Granola bar, high fiber, coated with non-chocolate yogurt coating
537	Bars	53712200	Granola bar, low-fat, NFS
537	Bars	53712100	Granola bar, NFS
537	Bars	53712210	Granola bar, nonfat
537	Bars	53714230	Granola bar, oats, nuts, coated with non-chocolate coating
537	Bars	53713100	Granola bar, peanuts, oats, sugar, wheat germ
537	Bars	53713000	Granola bar, reduced sugar, NFS
537	Bars	53714210	Granola bar, with coconut, chocolate-coated
537	Bars	53714400	Granola bar, with rice cereal
537	Bars	53710800	Kashi GOLEAN Chewy Bars
537	Bars	53710804	Kashi GOLEAN Crunchy Bars
537	Bars	53710802	Kashi TLC Chewy Granola Bar
537	Bars	53710806	Kashi TLC Crunchy Granola Bar
537	Bars	53710500	Kellogg's Nutri-Grain Cereal Bar
537	Bars	53710504	Kellogg's Nutri-Grain Fruit and Nut Bar
537	Bars	53710502	Kellogg's Nutri-Grain Yogurt Bar
537	Bars	53710700	Kellogg's Special K bar
537	Bars	53710600	Milk 'n Cereal bar
537	Bars	53710902	Nature Valley Chewy Granola Bar with Yogurt Coating
537	Bars	53710900	Nature Valley Chewy Trail Mix Granola Bar
537	Bars	53710906	Nature Valley Crunchy Granola Bar
537	Bars	53710904	Nature Valley Sweet and Salty Granola Bar
537	Bars	53711004	Quaker Chewy 25% Less Sugar Granola Bar
537	Bars	53711002	Quaker Chewy 90 Calorie Granola Bar

537	Bars	53711006	Quaker Chewy Dipps Granola Bar
537	Bars	53711000	Quaker Chewy Granola Bar
537	Bars	53711100	Quaker Granola Bites
537	Bars	53712000	Snack bar, oatmeal
537	Bars	53720100	Balance Original Bar
537	Bars	53720200	Clif Bar
537	Bars	53720210	Clif Kids Organic Zbar
537	Bars	53729000	Nutrition bar or meal replacement bar, NFS
537	Bars	53720300	PowerBar
537	Bars	53720400	Slim Fast Original Meal Bar
537	Bars	53720500	Snickers Marathon Protein bar
537	Bars	53720610	South Beach Living High Protein Bar
537	Bars	53720600	South Beach Living Meal Bar
537	Bars	53720700	Tiger's Milk bar
537	Bars	53720800	Zone Perfect Classic Crunch nutrition bar
612	Citrus fruit juices	61201020	Grapefruit juice, 100%, NS as to form
612	Citrus fruit juices	61201220	Grapefruit juice, 100%, canned, bottled or in a carton
612	Citrus fruit juices	61201620	Grapefruit juice,100%, frozen, reconstituted
612	Citrus fruit juices	61204200	Lemon juice, 100%, canned or bottled
612	Citrus fruit juices	61204000	Lemon juice, 100%, NS as to form
612	Citrus fruit juices	61207200	Lime juice, 100%, canned or bottled
612	Citrus fruit juices	61207000	Lime juice, 100%, NS as to form
612	Citrus fruit juices	61210000	Orange juice, 100%, NFS
612	Citrus fruit juices	61210220	Orange juice, 100%, canned, bottled or in a carton
612	Citrus fruit juices	61210250	Orange juice, 100%, with calcium added, canned, bottled or in a carton
612	Citrus fruit juices	61210820	Orange juice, 100%, with calcium added, frozen, reconstituted
612	Citrus fruit juices	61210720	Orange juice, 100%, frozen, not reconstituted
612	Citrus fruit juices	61210620	Orange juice, 100%, frozen, reconstituted
612	Citrus fruit juices	61213220	Tangerine juice, 100%
612	Citrus fruit juices	61213800	Fruit juice blend, citrus, 100% juice
612	Citrus fruit juices	61213900	Fruit juice blend, citrus, 100% juice, with calcium added
612	Citrus fruit juices	61201225	Grapefruit juice, 100%, with calcium added
631	Fruits, excluding berries	63143010	Plum, raw
631	Fruits, excluding berries	63143650	Plum, pickled
641	Fruit juices, excluding citrus	64101010	Apple cider
641	Fruit juices, excluding citrus	64104010	Apple juice, 100%
641	Fruit juices, excluding citrus	64104030	Apple juice, 100%, with calcium added
641	Fruit juices, excluding citrus	64104600	Blackberry juice, 100%
641	Fruit juices, excluding citrus	64100200	Cranberry juice blend, 100% juice

641	Fruit juices, excluding citrus	64100220	Cranberry juice blend, 100% juice, with calcium added
641	Fruit juices, excluding citrus	64105400	Cranberry juice, 100%, not a blend
641	Fruit juices, excluding citrus	64100110	Fruit juice blend, 100% juice
641	Fruit juices, excluding citrus	64100100	Fruit juice, NFS
641	Fruit juices, excluding citrus	64134030	Fruit smoothie juice drink (no dairy)
641	Fruit juices, excluding citrus	64134200	Fruit smoothie, bottled
641	Fruit juices, excluding citrus	64134100	Fruit smoothie, light
641	Fruit juices, excluding citrus	64134015	Fruit smoothie, with whole fruit (no dairy)
641	Fruit juices, excluding citrus	64134020	Fruit smoothie, with whole fruit (no dairy), added protein
641	Fruit juices, excluding citrus	64116020	Grape juice, 100%
641	Fruit juices, excluding citrus	64116060	Grape juice, 100%, with calcium added
641	Fruit juices, excluding citrus	64120010	Papaya juice, 100%
641	Fruit juices, excluding citrus	64121000	Passion fruit juice, 100%
641	Fruit juices, excluding citrus	64124020	Pineapple juice, 100%
641	Fruit juices, excluding citrus	64126000	Pomegranate juice, 100%
641	Fruit juices, excluding citrus	64132010	Prune juice, 100%
641	Fruit juices, excluding citrus	64132500	Strawberry juice, 100%
641	Fruit juices, excluding citrus	64133100	Watermelon juice, 100%
642	Nectars	64201010	Apricot nectar
642	Nectars	64201500	Banana nectar
642	Nectars	64202010	Cantaloupe nectar
642	Nectars	64200100	Fruit nectar, NFS
642	Nectars	64203020	Guava nectar
642	Nectars	64204010	Mango nectar
642	Nectars	64210010	Papaya nectar
642	Nectars	64213010	Passion fruit nectar
642	Nectars	64205010	Peach nectar
642	Nectars	64215010	Pear nectar
642	Nectars	64221010	Soursop (Guanabana) nectar
731	Carrots	73105010	Carrot juice, 100%
743	Tomato juices	74303000	Tomato and vegetable juice, 100%
743	Tomato juices	74303100	Tomato and vegetable juice, 100%, low sodium
743	Tomato juices	74302000	Tomato juice cocktail
743	Tomato juices	74301100	Tomato juice, 100%

743	Tomato juices	74301150	Tomato juice, 100%, low sodium
751	Other vegetables, raw	75132000	Mixed vegetable juice (vegetables other than tomato)
781	Vegetable and fruit juice blends, 100% juice	78101000	Vegetable and fruit juice, 100% juice, with high vitamin C
811	Table fats	81103040	Margarine-like spread, stick, salted
811	Table fats	81103041	Margarine-like spread, made with yogurt, stick, salted
811	Table fats	81103080	Margarine-like spread, tub, salted
811	Table fats	81103090	Margarine-like spread, liquid, salted
811	Table fats	81103100	Margarine-like spread, stick, unsalted
811	Table fats	81103120	Margarine-like spread, tub, unsalted
811	Table fats	81103130	Margarine-like spread, whipped, tub, salted
811	Table fats	81103140	Margarine-like spread, tub, sweetened
811	Table fats	81104011	Margarine-like spread, reduced calorie, about 40% fat, made with yogurt, tub, salted
811	Table fats	81104020	Margarine-like spread, reduced calorie, about 40% fat, stick, salted
811	Table fats	81104010	Margarine-like spread, reduced calorie, about 40% fat, tub, salted
811	Table fats	81104050	Margarine-like spread, reduced calorie, about 20% fat, tub, salted
811	Table fats	81104100	Margarine-like spread, fat free, tub, salted
811	Table fats	81104110	Margarine-like spread, fat free, liquid, salted
811	Table fats	81104550	Vegetable oil-butter spread, reduced calorie, stick, salted
811	Table fats	81104560	Vegetable oil-butter spread, reduced calorie, tub, salted
811	Table fats	81104500	Vegetable oil-butter spread, stick, salted
811	Table fats	81104510	Vegetable oil-butter spread, tub, salted
821	Vegetable oils	82104000	Olive oil
917	Candies	91705300	Chocolate, sweet or dark
917	Candies	91745010	Gumdrops
917	Candies	91745020	Hard candy
917	Candies	91770030	Dietetic or low-calorie candy, chocolate covered
917	Candies	91770000	Dietetic or low-calorie candy, NFS
917	Candies	91770010	Dietetic or low-calorie gumdrops
917	Candies	91770020	Dietetic or low-calorie hard candy
918	Chewing gums	91802000	Chewing gum, sugar free
923	Tea	92306100	Corn beverage
923	Tea	92307500	Iced Tea / Lemonade juice drink
923	Tea	92307520	Iced Tea / Lemonade juice drink, diet
923	Tea	92307510	Iced Tea / Lemonade juice drink, light
923	Tea	92306800	Tea, hot, chai, with milk
923	Tea	92306700	Tea, hot, chamomile
923	Tea	92306000	Tea, hot, herbal
923	Tea	92306090	Tea, hot, hibiscus
923	Tea	92302000	Tea, hot, leaf, black
923	Tea	92302500	Tea, hot, leaf, black, decaffeinated

923	Tea	92303010	Tea, hot, leaf, green
923	Tea	92303100	Tea, hot, leaf, green, decaffeinated
923	Tea	92304100	Tea, hot, leaf, oolong
923	Tea	92309000	Tea, iced, bottled, black
923	Tea	92309010	Tea, iced, bottled, black, decaffeinated
923	Tea	92309030	Tea, iced, bottled, black, decaffeinated, diet
923	Tea	92309050	Tea, iced, bottled, black, decaffeinated, unsweetened
923	Tea	92309020	Tea, iced, bottled, black, diet
923	Tea	92309040	Tea, iced, bottled, black, unsweetened
923	Tea	92309500	Tea, iced, bottled, green
923	Tea	92309510	Tea, iced, bottled, green, diet
923	Tea	92309520	Tea, iced, bottled, green, unsweetened
923	Tea	92308040	Tea, iced, brewed, black, decaffeinated, pre-sweetened with low calorie sweetener
923	Tea	92308030	Tea, iced, brewed, black, decaffeinated, pre-sweetened with sugar
923	Tea	92308050	Tea, iced, brewed, black, decaffeinated, unsweetened
923	Tea	92308010	Tea, iced, brewed, black, pre-sweetened with low calorie sweetener
923	Tea	92308000	Tea, iced, brewed, black, pre-sweetened with sugar
923	Tea	92308020	Tea, iced, brewed, black, unsweetened
923	Tea	92308540	Tea, iced, brewed, green, decaffeinated, pre-sweetened with low calorie sweetener
923	Tea	92308530	Tea, iced, brewed, green, decaffeinated, pre-sweetened with sugar
923	Tea	92308550	Tea, iced, brewed, green, decaffeinated, unsweetened
923	Tea	92308510	Tea, iced, brewed, green, pre-sweetened with low calorie sweetener
923	Tea	92308500	Tea, iced, brewed, green, pre-sweetened with sugar
923	Tea	92308520	Tea, iced, brewed, green, unsweetened
923	Tea	92305110	Tea, iced, instant, black, decaffeinated, pre-sweetened with low calorie sweetener
923	Tea	92305050	Tea, iced, instant, black, decaffeinated, pre-sweetened with sugar
923	Tea	92305180	Tea, iced, instant, black, decaffeinated, unsweetened
923	Tea	92305090	Tea, iced, instant, black, pre-sweetened with low calorie sweetener
923	Tea	92305040	Tea, iced, instant, black, pre-sweetened with sugar
923	Tea	92307400	Tea, iced, instant, black, pre-sweetened, dry
923	Tea	92305010	Tea, iced, instant, black, unsweetened
923	Tea	92307000	Tea, iced, instant, black, unsweetened, dry
923	Tea	92305920	Tea, iced, instant, green, pre-sweetened with low calorie sweetener
923	Tea	92305910	Tea, iced, instant, green, pre-sweetened with sugar

923	Tea	92305900	Tea, iced, instant, green, unsweetened
924	Soft drinks, carbonated	92410110	Carbonated water, sweetened
924	Soft drinks, carbonated	92410250	Carbonated water, sweetened, with low-calorie or no-calorie sweetener
924	Soft drinks, carbonated	92410210	Carbonated water, unsweetened
924	Soft drinks, carbonated	92432000	Fruit juice drink, citrus, carbonated
924	Soft drinks, carbonated	92433000	Fruit juice drink, noncitrus, carbonated
924	Soft drinks, carbonated	92410810	Soft drink, chocolate flavored
924	Soft drinks, carbonated	92410820	Soft drink, chocolate flavored, diet
924	Soft drinks, carbonated	92410310	Soft drink, cola
924	Soft drinks, carbonated	92411520	Soft drink, cola, chocolate flavored
924	Soft drinks, carbonated	92411620	Soft drink, cola, chocolate flavored, diet
924	Soft drinks, carbonated	92410340	Soft drink, cola, decaffeinated
924	Soft drinks, carbonated	92410350	Soft drink, cola, decaffeinated, diet
924	Soft drinks, carbonated	92410320	Soft drink, cola, diet
924	Soft drinks, carbonated	92411510	Soft drink, cola, fruit or vanilla flavored
924	Soft drinks, carbonated	92411610	Soft drink, cola, fruit or vanilla flavored, diet
924	Soft drinks, carbonated	92410315	Soft drink, cola, reduced sugar
924	Soft drinks, carbonated	92410410	Soft drink, cream soda
924	Soft drinks, carbonated	92410420	Soft drink, cream soda, diet
924	Soft drinks, carbonated	92410550	Soft drink, fruit flavored, caffeine containing
924	Soft drinks, carbonated	92410560	Soft drink, fruit flavored, caffeine containing, diet
924	Soft drinks, carbonated	92410510	Soft drink, fruit flavored, caffeine free
924	Soft drinks, carbonated	92410520	Soft drink, fruit flavored, diet, caffeine free
924	Soft drinks, carbonated	92410610	Soft drink, ginger ale
924	Soft drinks, carbonated	92410620	Soft drink, ginger ale, diet
924	Soft drinks, carbonated	92400000	Soft drink, NFS
924	Soft drinks, carbonated	92400100	Soft drink, NFS, diet
924	Soft drinks, carbonated	92410360	Soft drink, pepper type
924	Soft drinks, carbonated	92410390	Soft drink, pepper type, decaffeinated
924	Soft drinks, carbonated	92410400	Soft drink, pepper type, decaffeinated, diet
924	Soft drinks, carbonated	92410370	Soft drink, pepper type, diet
924	Soft drinks, carbonated	92410710	Soft drink, root beer
924	Soft drinks, carbonated	92410720	Soft drink, root beer, diet
925	Fruit drinks	92512050	Frozen daiquiri mix, from frozen concentrate, reconstituted
925	Fruit drinks	92512040	Frozen daiquiri mix, frozen concentrate, not reconstituted
925	Fruit drinks	92511015	Fruit flavored drink
925	Fruit drinks	92513000	Fruit flavored smoothie drink, frozen (no dairy)
925	Fruit drinks	92513010	Fruit flavored smoothie drink, frozen, light (no dairy)
925	Fruit drinks	92511250	Fruit juice beverage, 40-50% juice, citrus
925	Fruit drinks	92510610	Fruit juice drink
925	Fruit drinks	92510720	Fruit punch, made with fruit juice and soda
925	Fruit drinks	92510730	Fruit punch, made with soda, fruit juice, and sherbet or ice cream
925	Fruit drinks	92511000	Lemonade, frozen concentrate, not reconstituted

925	Fruit drinks	92510960	Lemonade, fruit flavored drink
925	Fruit drinks	92510955	Lemonade, fruit juice drink
925	Fruit drinks	92512110	Margarita mix, nonalcoholic
925	Fruit drinks	92512090	Pina Colada, nonalcoholic
925	Fruit drinks	92510650	Tamarind drink (Refresco de tamarindo)
925	Fruit drinks	92530510	Cranberry juice drink, with high vitamin C
925	Fruit drinks	92530410	Fruit flavored drink, with high vitamin C
925	Fruit drinks	92530610	Fruit juice drink, with high vitamin C
925	Fruit drinks	92531030	Sunny D
925	Fruit drinks	92530950	Vegetable and fruit juice drink, with high vitamin C
925	Fruit drinks	92541010	Fruit flavored drink, powdered, reconstituted
925	Fruit drinks	92542000	Fruit flavored drink, with high vitamin C, powdered, reconstituted
925	Fruit drinks	92550360	Apple juice beverage, 40-50% juice, light
925	Fruit drinks	92552030	Capri Sun, fruit juice drink
925	Fruit drinks	92550110	Cranberry juice drink, with high vitamin C, light
925	Fruit drinks	92550620	Fruit flavored drink, diet
925	Fruit drinks	92552010	Fruit flavored drink, powdered, reconstituted, diet
925	Fruit drinks	92550610	Fruit flavored drink, with high vitamin C, diet
925	Fruit drinks	92552000	Fruit flavored drink, with high vitamin C, powdered, reconstituted, diet
925	Fruit drinks	92550040	Fruit juice drink, diet
925	Fruit drinks	92550035	Fruit juice drink, light
925	Fruit drinks	92550030	Fruit juice drink, with high vitamin C, light
925	Fruit drinks	92550200	Grape juice drink, light
925	Fruit drinks	92550370	Lemonade, fruit juice drink, light
925	Fruit drinks	92550350	Orange juice beverage, 40-50% juice, light
925	Fruit drinks	92550380	Pomegranate juice beverage, 40-50% juice, light
925	Fruit drinks	92552020	Sunny D, reduced sugar
925	Fruit drinks	92550400	Vegetable and fruit juice drink, with high vitamin C, diet
925	Fruit drinks	92550405	Vegetable and fruit juice drink, with high vitamin C, light
925	Fruit drinks	92582100	Fruit juice drink, with high vitamin C, plus added calcium
925	Fruit drinks	92582110	Sunny D, added calcium
942	Water, bottled, fortified	94210200	Glaceau Vitamin Water
942	Water, bottled, fortified	94220215	Glaceau Vitamin Water Zero
942	Water, bottled, fortified	94210100	Propel Water
942	Water, bottled, fortified	94220110	Propel Zero Calcium Water
942	Water, bottled, fortified	94220100	Propel Zero Water
942	Water, bottled, fortified	94210300	SoBe Life Water
942	Water, bottled, fortified	94220310	SoBe Life Water Zero
951	Nutrition drinks	95101010	Boost Plus, nutritional drink, ready-to-drink
951	Nutrition drinks	95101000	Boost, nutritional drink, ready-to-drink
951	Nutrition drinks	95102000	Carnation Instant Breakfast, nutritional drink, regular, ready-to-drink

951	Nutrition drinks	95103010	Ensure Plus, nutritional shake, ready-to-drink
951	Nutrition drinks	95103000	Ensure, nutritional shake, ready-to-drink
951	Nutrition drinks	95104000	Glucerna, nutritional shake, ready-to-drink
951	Nutrition drinks	95105000	Kellogg's Special K Protein Shake
951	Nutrition drinks	95106010	Muscle Milk, light, ready-to-drink
951	Nutrition drinks	95106000	Muscle Milk, ready-to-drink
951	Nutrition drinks	95120020	Nutritional drink or meal replacement, high protein, light, ready-to-drink, NFS
951	Nutrition drinks	95120010	Nutritional drink or meal replacement, high protein, ready-to-drink, NFS
951	Nutrition drinks	95120050	Nutritional drink or meal replacement, liquid, soy-based
951	Nutrition drinks	95120000	Nutritional drink or meal replacement, ready-to-drink, NFS
951	Nutrition drinks	95110020	Slim Fast Shake, meal replacement, high protein, ready-to-drink
951	Nutrition drinks	95110000	Slim Fast Shake, meal replacement, regular, ready-to-drink
951	Nutrition drinks	95110010	Slim Fast Shake, meal replacement, sugar free, ready-to-drink
952	Nutrition powders	95201000	Carnation Instant Breakfast, nutritional drink mix, regular, powder
952	Nutrition powders	95201010	Carnation Instant Breakfast, nutritional drink mix, sugar free, powder
952	Nutrition powders	95201300	EAS Soy Protein Powder
952	Nutrition powders	95201200	EAS Whey Protein Powder
952	Nutrition powders	95201500	Herbalife, nutritional shake mix, high protein, powder
952	Nutrition powders	95201600	Isopure protein powder
952	Nutrition powders	95201700	Kellogg's Special K20 Protein Water Mix
952	Nutrition powders	95202010	Muscle Milk, light, powder
952	Nutrition powders	95202000	Muscle Milk, regular, powder
952	Nutrition powders	95220010	Nutritional drink mixes or meal replacement, high protein, powder, NFS
952	Nutrition powders	95220000	Nutritional drink mix or meal replacement, powder, NFS
952	Nutrition powders	95230020	Protein powder, light, NFS
952	Nutrition powders	95230030	Protein powder, NFS
952	Nutrition powders	95230010	Protein powder, soy based, NFS
952	Nutrition powders	95230000	Protein powder, whey based, NFS
952	Nutrition powders	95210020	Slim Fast Shake Mix, high protein, powder
952	Nutrition powders	95210000	Slim Fast Shake Mix, powder
952	Nutrition powders	95210010	Slim Fast Shake Mix, sugar free, powder
9532	Sports drinks	95320200	Gatorade G sports drink
9532	Sports drinks	95322200	Gatorade G2 sports drink, low calorie
9532	Sports drinks	95320500	Powerade sports drink
9532	Sports drinks	95322500	Powerade Zero sports drink, low calorie
9532	Sports drinks	95323000	Sports drink, low calorie
9532	Sports drinks	95321000	Sports drink, NFS

BioActor B.V.
Maastricht Health Campus
Gaetano Martinolaan 50,
6229 GS Maastricht
The Netherlands

Maastricht, August 28, 2023

To the attention of Jason Downey, Ph.D.

Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration

Re: GRN 001119 – FDA Questions to the Notifier

Dear,

Please find hereby the answers that we prepared in response to the set of FDA Questions to the Notifier that we received July 21, 2023.

Sincerely,

Hans van der Saag
Managing Director

GRN 001119 – Answers to FDA Questions to the Notifier

Date: August 28, 2023

1. **In part 1 of your notice, you state part 1 contains "personal privacy information." Please explain which if any sections of part 1 of the notice you believe are exempt from disclosure under the Freedom of Information Act.**

   None of the sections in Part 1 is exempt from disclosure, consequently BioActor BV takes out the refence in Paragraph 1.8.

2. **We have the following questions related to the manufacturing process and specifications for olive leaf extract:**
   a. **On pages 9 and 10 of the notice, you provide general information about the manufacturing process of the ingredient, along with a manufacturing flowchart (Figure 3). Please provide a more detailed narrative of the manufacturing process that coincides with the provided flowchart**.

      The manufacturing process of Bonolive® is described as follows:
      - Healthy olive leaves are pruned from olive trees grown in Spain, specifically in the Andalusia region. Such pruning typically takes place during the month of February. In fact, even if the trees are pruned twice a year, in February and August, the leaves collected in August are not utilized as they may have a lower oleuropein content due to high temperatures in summer months.
      - The harvested leaves are cleaned and then dried under ambient conditions to reduce their moisture content. Once dried, the leaves are milled, to reduce the size and therefore increase the surface area, which can facilitate the extraction process.
      - Dried and ground olive leaves are extracted using a polar hydroalcoholic medium (ethanol + water), in a concentration of at least 70% by weight. The mixture is allowed to sit for at least 12 hours at room temperature, to allow the bioactive compounds to dissolve into the hydroalcoholic medium. This step may be repeated up to 3 times. Afterwards, the ethanol is evaporated.
      - The crude olive leaf extract is then filtered and purified via macroporous resin adsorption.
      - Afterwards, the extract is concentrated under vacuum at a temperature of about 60° to 90°C, and it is then treated at around 80-90°C for about 16-20 hours to reduce microbial load.
      - Finally, the concentrated extract is spray dried at a temperature of about 70°C to 90°C, to obtain a dry powder extract.
      - Before the final packaging step in polyethylene bags, the spray dried extract is milled and standardized. The product is analyzed to ensure it contains at least 40 weight percent oleuropein, and does not exceed 45 weight percent oleuropein.

   b. **On page 11 you state that olive leaf extract is manufactured according to Global Food Safety Initiative standards. Please confirm that olive leaf extract is manufactured according to current good manufacturing practices and that all materials used in the manufacture of the ingredient are used in accordance with current U. S. regulations, are considered GRAS for their intended use, or are the subject of an effective FCN.**

      BioActor BV confirms that the olive leaf extract Bonolive® is manufactured according to the 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. Consequently, the olive leaf extract is manufactured according to current GMP.
All materials used in the manufacture of the olive leaf extract are used in accordance with current U. S. regulations and it is considered GRAS for its intended use.
The packaging material also meets the requirements of FDA's applicable regulations.

c. You indicate that the olive leaf extract is a minimum of 50% polyphenols and that the remainder of the ingredient is carbohydrates, proteins, and minerals. Please provide the percent composition of these remaining components of olive leaf extract.

As described in table in 2.5.6, the essential nutrients found in Bonolive® are as follows:

Description	Result (%)	Unit
Total Fat Content	0.3	g/100g of product
Saturated fatty acids	56.4	% of fatty acids
Total Carbohydrates (including polyphenols)	94.4	g/100g of product
Assimilable carbohydrates	93.1	g/100g of product
Total Sugar (as Glucose)	4.2	g/100g of product
Total Fibers	1.3	g/100g of product
Total Protein	0.9	g/100g of product
Sodium	62.7	mg/100g of product

A distinction should be made between macronutrients and polyphenols: macronutrients content of Bonolive is set forth in table 2.5.6 (see above). Polyphenols, which account of 50% of the extract, are not considered macronutrients but non-nutrients. Text to be amended accordingly in Paragraph 2.2.1. However, it is customary to consider the total polyphenols as part of the total quantified carbohydrates.

d. In Table 2, you list the specifications and analytical methods for olive leaf extract. Please confirm that all methods of analysis are validated for their intended uses.

BioActor BV confirms that all the analytical methods are based on the European Pharmacopoeia (Ph.Eur.) and validated for their intended use. The methods outlined in the Ph. Eur. are validated for their intended uses. Moreover, the coliforms analysis is performed in accordance with the ISO 4832:2006 standard, which is a validated method for the detection and enumeration of coliforms in food and animal feeding stuffs.

e. In Table 3, you provide information for 4 batches of olive leaf extract and state that the ingredient "complies" with certain specifications. Please provide actual values for these specifications to demonstrate that the ingredient can consistently meet the set specifications. We also request that your specifications for heavy metals be as low as possible and are representative of the results of your batch analyses to align with FDA's Closer to Zero initiative of reducing dietary exposure to environmental contaminants from food. Please also indicate the limit of detection for the method used to analyze for heavy metals.

BioActor BV amended the specifications table as follows:

Test Items	Specification	Batch Number				
		PF0348220321	PF0600130720	PF1138260520	PF1043200320	PF1224240720

Appearance	Green to brown powder	Complies	Complies	Complies	Complies	Complies
Botanical part used	Olea Europaea L. (leaf)	Complies	Complies	Complies	Complies	Complies
Loss on drying	8% max	1.72%	2.05%	1.57%	2.06%	0.64%
Residue by calcination	9% max	0.5%	1.5%	1.1%	<9%	<9%
Total polyphenols	50% min	55.9%	51.6%	53.1%	54%	55.5%
Oleuropein	40% min	42.3%	40.9%	40.3%	40.04%	41.8%
Residual ethanol	100 ppm max	34.7 ppm	33 ppm	31.7 ppm	39.3 ppm	57.9 ppm
Heavy metals						
Lead	3 ppm max	< 0,10 ppm	< 0,10 ppm	< 0,10 ppm	< 0,10 ppm	< 0,10 ppm
Cadmium	1 ppm max	< 0.020 ppm	< 0.020 ppm	< 0.020 ppm	< 0.020 ppm	< 0.020 ppm
Mercury	0.1 ppm max	< 0.010 ppm	< 0.010 ppm	< 0.010 ppm	< 0.010 ppm	< 0.010 ppm
Arsenic	2 ppm max	0.38 ppm	< 0.020 ppm	< 0.020 ppm	< 0.020 ppm	< 0.020 ppm
PAHS						
Benzo(a)pyrene	10.0 ppb max	< 0.5 ppb	< 0.5 ppb	< 0.5 ppb	< 0.5 ppb	< 0.5 ppb
Sum of benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene and chrysene	50.0 ppb max	< 0.5 ppb	< 0.5 ppb	< 0.5 ppb	< 0.5 ppb	< 0.5 ppb
Microbiological Tests (*)						
Total Plate Count	10^3 cfu/g max	Complies	Complies	Complies	Complies	Complies
Yeast & Mold	10^2 cfu/g max	Complies	Complies	Complies	Complies	Complies
Enterobacteriaceae	10^2 cfu/g max	Complies	Complies	Complies	Complies	Complies
Escherichia coli	Absence/1 g	Complies	Complies	Complies	Complies	Complies
Salmonella	Absence/25 g	Complies	Complies	Complies	Complies	Complies
Staphylococcus aureus	Absence/g	Complies	Complies	Complies	Complies	Complies
Coliforms	Absence/g	Complies	Complies	Complies	Complies	Complies

The appearance parameter refers to a visual judgement of the batch and hence no value is available.
The botanical part used parameter is assessed through a visual inspection of the leaves and hence no value is available.
For the heavy metals results, the batch is compliant with the specification when the lead, cadmium, mercury, and arsenic quantification is below the LOQ (limit of quantification). LOQ for heavy metals are as follows:

Arsenic: 0,020 mg/kg
Cadmium: 0,020 mg/kg
Mercury: 0,010 mg/kg
Lead: 0,10 mg/kg

For the PAHs results, the batch is compliant with the specification when the benzo(a)pyrene and the sum of benzo(a)anthracene, benzo(b)fluoranthene and chrysene quantification is below the LOQ (limit of quantification). LOQ for PAHs are as follows:

Benzo(a)pyrene: 0,5 µg/Kg
Sum of benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene and chrysene: 0,5 µg/Kg

(*) As regards microbiological parameters, the batch is compliant with the specification when the resulting values are < 10^2 (LOQ), or when the target microorganism is absent.

f. Table 2 lists a specification for ethanol of 1000 mg/kg; however, the results of the batch analyses indicate that all batches were below 100 mg/kg. Please indicate why a specification one order of magnitude higher is needed.

BioActor BV agrees to lower the product specification for ethanol as a residual solvent to 100 mg/kg.
The residual ethanol parameter in the product specifications is amended to < 100 ppm in table 3 of Paragraph 2.5.1, as analysis results are expected to be always < 100 ppm.

3. You indicate that only certain cookies are included in the intended uses in Table 1 and Table 7. Please indicate what cookies are excluded from the scope of the intended uses.

BioActor BV's excluded the following cookie types from the scope of intended uses of Bonolive®:

Food code	Food name
53201000	Cookie, NFS
53202000	Cookie, almond
53220030	Cookie, fig bar
53220040	Cookie, fig bar, fat free
53223100	Cookie, granola
53231400	Cookie, multigrain, high fiber
53233000	Cookie, oatmeal
53233010	Cookie, oatmeal, with raisins
53233040	Cookie, oatmeal, reduced fat, NS as to raisins
53235600	Cookie, Pfeffernusse
53236100	Cookie, pumpkin
53237000	Cookie, raisin
53239010	Cookie, shortbread, reduced fat
53241510	Marie biscuit
53241600	Cookie, butter, or sugar, with fruit and/or nuts
53246000	Cookie, tea, Japanese
53247050	Cookie, vanilla wafer, reduced fat
53260200	Cookie, oatmeal, sugar free
53260400	Cookie, sugar or plain, sugar free
53260500	Cookie, sugar wafer, sugar free

4. In part 3 of the notice, you discuss the estimated dietary exposure to olive leaf extract based on the intended uses. You note that you used food consumption data from the U.S. National Health and Nutrition Examination Survey (NHANES) What We Eat in America but did not indicate which data set was used. Please provide the year(s) of the NHANES data used to estimate the dietary exposure.

The NHANES data set that was used to estimate the dietary exposure to olive leaf extract is from 2016 , as BioActor BV commissioned an exposure calculation several years ago when only the 2016 NHANES data set was available. Given the fact that food consumption data have not changed significantly in the past years and given the cost associated with accessing more recent NHANES data, BioActor BV decided to use the 2016 data set as a basis for this submission.

5. **In Table 6 you state that there are about 19% unquantified polyphenols in the ingredient. Question: Because these unquantified polyphenols belong to olive leaves, please discuss**
    a. **Whether these are commonly consumed olive polyphenols whose safety and metabolism are known.**
    b. **If these polyphenols are commonly consumed, where else are they found in addition to the olive leaves (e.g., Olive pulps? Other parts of olive tree? Other edible fruits?).**
    c. **Does this group of unidentified phenolics include any member that may have adverse effects?**

Our statement about 19% unidentified polyphenols should be understood to relate to the relative proportion of unidentified polyphenols against the total polyphenols content. Since the extract contains about 50% polyphenols, the % of unidentified polyphenols in the total extract is about 9,5%.

Table 6 is complemented by a polyphenol identification analysis that BioActor commissioned, published in a peer reviewed journal[1], providing a more complete identification of the 9.5% % polyphenols can be made. Figure 1 from this publication summarizes the different polyphenols that were found in Bonolive®.

Peak	Compounds	Retention time (min)	$[M-H]^-$	MS/MS
1	Hydroxytyrosol glucoside	11.65; 11.96	315	153
2	Oleoside	12.09	389	227, 209, 183
3	Luteolin diglucoside	21.99; 24.30	609	447, 285
4	Elenolic acid glucoside	24.61	403	241, 223, 162
5	Dimethyl-oleuropein glucoside	24.82	525	481, 389, 363, 347, 319, 209, 195
6	Hydroxy-oleuropein glucoside	25.64; 28.16	555	537, 403, 393, 323
7	Rutin	25.82	609	431, 301
8	Luteolin rutinoside	26.15	593	447, 285
9	Verbascoside	26.83; 28.46	623	461, 315
10	Luteolin glucoside	27.02; 30.21	447	285
11	Apigenin rutinoside	28.82	577	269
12	Luteolin rutinoside	29.20	593	285
13	Oleuropein diglucoside	29.50; 30.82	701	539, 377
14	Chrysoeriol glucoside	31.08	461	446, 299
15	Oleuropein glucoside	32.68; 33.84; 34.46	539	437, 403, 377, 275

Figure 1. Polyphenols table (Garcīa-Villalba et al., 2013)

As expected, the most abundant polyphenol identified was oleuropein of which three different isomers but with the same molecular weight and mass spectrometric daughter ions. The other oleuropein derivatives found were oleoside, dimethyl-oleuropein and hydroxy-oleuropein and a low quantity of hydroxytyrosol glucoside and elenolic acid glucoside. In addition tot he oleuropein isomers and derivatives, Figure 1 also lists the other polyphenols that are quantified and listed in Table 6 (i.e. Verbascoside, Luteolin and its glycosides). In addition to Table 6, Elenolic acid (precursor of Oleuropein), Rutin (glycosylated form of Quercetin), Apigenin and Chrysoeriol (Luteolin derivative) are listed in Table 6.

A. B. According to literature, all the identified phenolic compounds are the same type of the ones found in olive fruits, which are commonly used for human consumption. [2]

C. None of the polyphenols identified in Bonolive® have shown an adverse effect, as Bonolive® was used as the test substance for the toxicological study performed by Clewell et al.[3] Therefore, all polyphenols that are part of the extract were tested for safety: the consumption of Bonolive® via gavage for up to 90 days did not result in adverse effects in CRL:(WI)BR Wistar rats.

Cited Literature:

1. García-Villalba R, Larrosa M, Possemiers S, Tomás-Barberán FA, Espín JC. Bioavailability of phenolics from an oleuropein-rich olive (Olea europaea) leaf extract and its acute effect on plasma antioxidant status: comparison between pre- and postmenopausal women. Eur J Nutr. 2014 Jun;53(4):1015-27. doi: 10.1007/s00394-013-0604-9. Epub 2013 Oct 26. PMID: 24158653)
2. El SN, Karakaya S. Olive tree (Olea europaea) leaves: potential beneficial effects on human health. Nutr Rev. 2009 Nov;67(11):632-8.
3. Clewell AE, Béres E, Vértesi A, Glávits R, Hirka G, Endres JR, Murbach TS, Szakonyiné IP., 2016. A Comprehensive Toxicological Safety Assessment of an Extract of Olea Europaea L. Leaves (Bonolive™). Int J Toxicol. 35(2):208-21. doi: 10.1177/1091581815619764.

6. You discussed pharmacokinetic studies of olive leaf extracts.

Question:

a. **Please provide a brief discussion of the ADME of olive tree polyphenols with special emphasis on oleuropein from two recent publications that are not cited in your GRAS notice (Galmes et al. *J Agric Food Chem* 2021; 69(18): 5281-5296. doi: 10.1021/acs.jafc.1c00737; Nikou et al. *Nutrients* 2022, 14, 3773. https://doi.org/10.3390/nu14183773).**

b. **Please state whether there is any information in these publications that could contradict your conclusions on the safety and ADME of olive leaf polyphenols or does the information in these publications corroborate your conclusions.**

Thank you for bring to our attention the additional recent publications. We have extensively described both these publication below along with our comments.

a. **Galmés S, Reynés B, Palou M, Palou-March A, Palou A., 2021. Absorption, Distribution, Metabolism, and Excretion of the Main Olive Tree Phenols and Polyphenols: A Literature Review. J Agric Food Chem. 69(18):5281-5296.**

This review article described studies on the administration of hydroxytyrosol (HT), oleuropein (Ole), or other olive tree polyphenols and foods, products, or mixtures that contain them. The authors selected 45 original articles, based on their collection criteria, and discussed and evaluated them for this review. This review addressed the absorption, distribution, metabolism, and excretion (ADME) processes of olive tree polyphenols applying the methods and criteria discussed in the article. Based on their review criteria, the authors provided their concluding remarks as quoted below. Please note that the numbers in the concluding remarks are the reference numbers cited in the review document.

We accept the following summation by the authors.

The effects of olive tree (poly)phenols (OPs) are largely dependent upon their bioavailability and metabolization by humans. ADME are fundamental for the nutritional efficacy and toxicological impact of foods containing OPs. This review includes studies on the administration of hydroxytyrosol (HT), oleuropein (Ole), or other OPs and foods, products, or mixtures that contain them. Briefly, data from *in vivo* studies indicate that OPs are absorbable by intestinal cells. Both absorption and bioavailability depend upon each compound and/or the matrix in which it is contained. OPs metabolism begins in enterocytes and can also continue in the liver. Metabolic phase I mainly consists of the hydrolysis of Ole, which results in an increase in the HT content. Phase II metabolic reactions involve the conjugation of (poly)phenols mainly with glucuronide and sulfate groups. This review offers a complete perspective of the ADME processes of OPs, which could support the future nutritional and/or toxicological studies in this area.

Concluding author remarks

"Data on ADME in humans and animals are important for the assessment of both the nutritional and toxicological impact of foods containing bioactive compounds. A considerable amount of information has been published regarding ADME of OPs and foods and other matrices containing OPs, either naturally or artificially. In this regard, most of the aforementioned studies describe the ADME processes of the main OP, HT, and Ole or foods/products rich in these compounds, which are briefly recapitulated below and summarized in Table 1 (considering animal studies) and Table 2 (considering human studies). Absorption of HT and Ole has been specifically characterized in three animal and six human studies, which pointed out a rapid absorption of HT. In animals, HT can be detected between 5 and 15 min after administration, peaking after 30 min, and other HT metabolites have also

been observed in plasma, such as HVAlc and HVA.[13−15] In humans, HT is also absorbed quickly, with peak times from 13 to 120 min after administration, depending upon the doses and form of treatment.[8,16,18,19,21,23] The higher differences on the absorption time (13−120 min) of OP in humans can be probably due to the diversity in their methodology. For example, the studies by Kountouri et al.[19] and Miró-Casas et al.[20] assess absorption from different matrices (olives and olive oil) in experimental samples with very few subjects, with 7 and 6 people, respectively. On the other hand, the study carried out by Rubióet al.[22] also tested the absorption of raw VOO, but even its study has been performed with an acceptable number of subjects, with the results obtained not comparable to the general population, because volunteers had hypercholesterolemia.

Moreover, recent investigations pinpoint that the food matrix is also determinant for the absorption of HT.[24] Meanwhile, Ole shows poor intestinal absorption as a result of its hydrophobic characteristics and higher molecular weight, showing greater stability under digestion conditions and lower in vivo absorption rates than HT.[15,17] In humans, Ole metabolites are detected in plasma after the ingestion of olive leaf extract supplements,[8] which peaked in plasma at 23 and 38 min after treatment. This lower bioavailability of Ole could probably be explained because it requires alternative uptake mechanisms than HT[11] and/or needs microflora activity to be absorbed.[10] Despite this, in vivo studies endorse the distribution of Ole derivatives to different tissues and organs of animals after ingestion of olive pomace.[17] Then, Ole can be hydrolyzed and converted to HT by phase I metabolism reactions in enterocytes, causing increases in the plasma HT content, at the expense of Ole.[8,20,21,23,28,29]

The distribution of OPs has been characterized in four in vivo studies in rodents,[13,17,25,26] which displayed that OP compounds reach tissues and organs, such as the heart, brain, liver, kidney, spleen, testes, thymus, and red blood cells, a few minutes after injection.[13,25] No study has been found regarding distribution in humans. This could be due to the technical limitations to carry out this type of approach; therefore, it would be necessary to carry out trials with new methodologies to better characterize this process in humans.

The metabolism of OPs may start at once in the enterocyte (and/or the metabolism could be completed in liver) after the absorption process. Part of ingested Ole is hydrolyzed to HT; therefore, phase I metabolic reactions result in an increase in the HT content.[8] Otherwise, ingested HT is rapidly metabolized through phase I metabolic reactions. Then, HT or HT derived from Ole is conjugated with sulfate or glucuronide groups by phase II metabolic reactions.[33] The metabolites resulting from phase I and phase II OP reactions are diverse and may depend upon the matrix ingested, the dose administered, and the model studied.[20,30,32,33] For instance, in humans, over 50 phase I and II metabolites were detected in urine at 2 h after intake of 50 mL of EVOO intake.[45]

Finally, OP excretion is performed via the kidneys through urine.[46] In this context, *in vivo* studies contribute to characterizing this process in rodents, where the excretion percentage may depend upon the administration method, the OP administered, and the dose. In rats, 70.9−94.9% of HT is recovered in urine at 24 h after treatment, and the same occurs with Tyr, with 53.2−74.4% recovery of the total administrated.[39] On the other hand, the administration of Ole is associated with higher excretion amounts of phase I and II metabolic reaction products, i.e., HVA whose excretion could turn out to be 50-fold higher in rats treated with Ole than groups treated with HT or other OPs.[14] In humans, HT seems to be mainly excreted as HVA, DOPAC glucuronide, DOPAC, HVA sulfate, HVA glucuronide, and DOPAC sulfate.[18] In addition, the maximum peak of excretion in urine of OPs seems to be around 4 h after ingestion, with some differences depending upon the type of treatment studied and the metabolite analyzed.[19,22,40,41,43,47] In addition, the polarity of the supplement seems to mildly influence the excretion of OP- rich supplements.[42]

In conclusion, there is relevant information regarding ADME of the main OPs, shown through both animal and human studies. In these studies, diverse OPs were analyzed using different concentrations, tested by themselves or included in foods (i.e., VOO) or other matrices (olive

byproducts, olive leaf extracts, or nutraceuticals). Generally, OPs are bioavailable by intestinal cells and are generally absorbed in a dose-dependent manner. Then, some phase I or II metabolic reactions occur during passage through the enterocyte. Some of these reactions can also occur in the liver. Thereafter, distribution through the bloodstream takes place, and OPs can reach various tissues and organs, before being excreted in urine. The maximum excretion peak occurs during the first 8 h after ingestion and can reach 24 h. Finally, some results in human studies show high variability in OP absorption, metabolism, and excretion processes, which could be explained by factors including differences in food matrices, differential phenolic composition of tested compounds, and/or interindividual differences of consumers (gender, enzyme activity, or genetics)."

BioActor BV's Comments

It is difficult to correlate this review article with olive leaf extracts.

While it is recognized that many of the phenols and polyphenols in olive trees may also be present in olive leaves, unless these compounds are specifically identified and their concentrations determined, and this data compared side-by-side, the relevance is diminished. Based on our review, we do not see any information in these publications that could contradict conclusions on the safety and ADME of olive leaf polyphenols found in the olive leaf extract, primarily oleuropein (76%) and other minor like verbascoside and luteolin. The information in these publications corroborate the safety determination. We accept the authors statement that "there is relevant information regarding ADME of the main OPs shown through both animal and human studies." We also agree that additional studies are needed in this area. As described in the GRAS notice, the safety of olive leaf extract is based on the totality of available evidence that includes a ninety-day repeat dose oral toxicity study, on the subject of the GRAS, in rats.

b. Nikou, Theodora, Maria Eleni Sakavitsi, Evangelos Kalampokis, and Maria Halabalaki. "Metabolism and bioavailability of olive bioactive constituents based on in vitro, in vivo and human studies." *Nutrients* 14, no. 18 (2022): 3773.

This review focused mainly on the metabolism and bioavailability of olive bioactive compounds through in vitro, in vivo, and human studies. In this recent review, the authors stated that the "… critical review summarizes the existing knowledge regarding the bioavailability and metabolism of olive-characteristic phenylalcohols and secoiridoids and spotlights the lack of data for specific chemical groups and compounds."

The authors devoted the first four pages of the review article discussing the issues and problems associated with research in this area. Based on their review criteria, the authors provided their concluding remarks as quoted below.

Concluding author remarks

"Currently, the bioavailability of food bioactives is studied as an integral part to the demonstration of a health benefit. Olive products, rich in OBs (biophenols), are among the most studied foodstuffs in terms of their chemical composition and health beneficial effects, whereas limited and scattered data exist for their bioavailability. In the current review, the author attempted to gather all the recent information regarding the bioavailability and metabolism of OBs. The effort targeted the most studied chemical groups of compounds regarding thei pharmacological properties, especially phenylalcohols and secoiridoids, namely HTyr, Ty Oleo, Olea and Oleu. The aim of the current approach was to highlight the basic metabolic differences of OBs according to their chemical structure. Special attention was also given on human gut microbiome metabolism, which currently prevails in contemporary research design. Based on the first scrutiny of the used literature, the majority of publications concentrate on HTyr, followed by Oleu, due to their commercial availability. As expected HTyr, phenolic extracts and enriched OO expose plentiful data in humans and

less in vitro and in vivo due to the ease of their administration. However, this fact is not usually found in the study of natural products' ADME properties. Our investigation revealed that regarding phenylalcohols, there is strong evidence for ADME(T) properties and there is a large amount of information regarding the bioavailability and metabolism of these compounds. Both HTyr and Tyr have been found to be absorbed in a dose-dependent manner in humans. A variation on results given for HTyr and Tyr is linked with the quantitation methodologies and their endogenous biosynthesis. At least 16 metabolic derivatives have been identified so far, while excretion levels T_{max} and C_{max} have been determined. Bioavailability is the only parameter under question due to analytical challenges existing for their detection in biological matrices.

On the other hand, fewer information was found for secoiridoids. The peculiar and sensitive chemical structure of secoiridoids hinders the design of metabolism studies due to isolation and detection difficulties of these compounds. The bioavailability of Olea and Oleo has been scarcely studied either in *in vitro* studies or in preclinical and clinical trials. Most research in this field regarding secoiridoids has been focused on Oleu and its aglycon forms. Oleu showed most of the data and witness possible biotransformations of the rest secoiridoids, yet all studies are focused on the phenylalcohol pathway, totally neglecting the elenolic part of the secoiridoids. Additionally, the lack of standard compounds has limited human intervention and is usually investigated as an extract or enriched OO. Generally, it can be presumed that *in vitro* outcomes can be used as first observations for the design and performance of in vivo experimentations, while the consequent human studies could give the go/no-go decision for the use of such compounds as nutraceuticals. However more research is required to draw conclusions about the bioavailability and metabolism of such sensitive and unstable compounds. Additionally, this could provide insight regarding extra virgin OO as a more complex food mixture with multiple beneficial health effects. Regarding OBs' colonic metabolism, limited studies were found and preliminary result around OBs' metabolism by human gut microbiota could inspire the design of future experimental approaches. Hence, the metabolism studies of OO and OBs specifically is a contemporary subject of research that could contribute significantly to the genera understanding of dietary interventions to prevent or even cure human malfunctions, guide personalized nutrition, inform toxicology risk assessment and improve drug discovery and development."

BioActor BV's comments

This review is interesting as it points out deficiencies and data gaps in this area. However, it did not point out what future experiments would be necessary going forward. The applicability of this review to olive leaf extracts is questionable. The information described in this article does not contradict the conclusions related to the safety and ADME of olive leaf polyphenols. The study on bioavailability of leaf extracts in which presence of the metabolites in urine was noted, indicates that oleuropein reaches systemic circulation and is metabolized in the human system. The studies described in this article indicate that olive leaf extract is unlikely to raise any safety concerns.

7. **A literature search by FDA retrieved two publications that described adverse reproductive effects of olive leaf extract (Najafizadeh et al. *Iran J Reprod Med*. 2013; 11(4): 293-300, PMID: 24639759; and Hakemi et al. *Int J Fertil Steril* 2019; 13(1): 57-65, doi: 10.22074/ijfs.2019.5520) as well as histopathological alterations in both liver and kidneys including elevation of clinical parameters in rats (Omer et al. *Asian J Animal Veterinary Advances* 2012 7 (11): 1175-1182, DOI: 10.3923/ajava.2012.1175.1182). These publications are not discussed in the notification.**
Question: It is important to note that publicly available toxicity information contradicts GRAS conclusion.

a. Therefore, please discuss these studies and explain why these reports of adverse effects of olive tree extract do not contradict your GRAS conclusion.

b. In this context, please reiterate with examples and citations whether olive leaf extracts have been traditionally consumed by humans, and whether the reported adverse effects in rats could be species-specific or triggered by the doses used or by some other mechanisms. Please cite published information to make your case in support of the GRAS conclusion of the dry extract of olive leaves. It is important to emphasize in this context that an explanation based on species-specific adverse effects might invalidate the utility of your pivotal toxicology studies and other similar studies conducted in rats.

Thank you for bringing to our attention the publication that revealed adverse effects of olive leaf extract. We have discussed these articles below along with some discussion and comments.

a.

1) **Najafizadeh, Parvaneh, Farzaneh Dehghani, Mohammadreza Panjeh Shahin, and Sommaye Hamzei Taj. "The effect of a hydro-alcoholic extract of olive fruit on reproductive argons in male sprague-dawley rat."** ***Iranian Journal of Reproductive Medicine*** **11, no. 4 (2013): 293.**

This study investigated the effects of olive fruit on the fertility reduction in male rats. The title: "The effect of a hydro-alcoholic extract of olive fruit on reproductive argons in male sprague-dawley rat" is confusing as the word "argons" should be "organs". It is surprising to note that neither authors of the reviewers noted this mistake in the title. The investigators conclude that this olive fruit extract may have deleterious effects on fertility factors. The available information on olive from its current and historical uses, as well as multiple scientific studies suggest that olive fruit or its preparation is unlikely to cause such effects.

In this study, 40 Sprague-Dawley male rats with the average weight of 200-250 grams and age of 8-10 weeks, were divided into 5 groups. The animals (eight per group) were administered by gavage (daily?) as follows: control (0 mg); vehicle (normal saline); 50, 150, and 450 mg/kg for 48 days. The test solution was an alcoholic (70%) extract of olive fruit. At the end of the study, the animals were sacrificed, and biochemical and anatomical results were obtained and tabulated. The results of this study are summarized below.

Results

Oral administration of various concentrations of olive extract resulted in no significant difference in the rats' weights among the control group, the vehicle, and the experimental groups. The weights of the left testicle in the groups administered dosages of 50, 150, and 450 mg/kg and seminal vesicle in the groups administered a dosage of 150 mg/kg showed a significant decrease ($p=0.03$). However, there was no noticeable difference with regard to the weights of prostate ($p=0.07$) and epididymis ($p=0.10$). The results of the measuring of the testosterone demonstrate a significant decrease ($p\leq0.04$) in testosterone in the experimental groups in comparison with the control group. The highest decrease was observed in the group administered the 450 mg/kg dosage.

The results of the measuring of the estradiol, reveal no significant difference among the control, vehicle and/or other experimental groups ($p\leq0.07$).

There was a significant decrease ($p\leq0.001$) in the sperm count of the groups administered dosages of 50, 150 and 450 mg/kg/day in comparison with the control and vehicle groups; the most effective dose was 450 mg/kg/day.

The results of the study of sperm motility show a significant decrease ($p \leq 0.04$) in the sperm motility of the groups administered dosages of 50, 150 and 450 mg/kg/day in comparison with the control and vehicle groups.

The results also show a significant decrease in testosterone level among the five groups, which is dependent on the concentration of the extract; the decrease in testosterone is positively correlated to the concentration of the extract.

The results of the study show no significant differences of estradiol levels among the groups.

The administration of the olive extract in all of the three concentrations resulted in a significant decrease in both sperm count and sperm motility. The authors stated that, "… it can be said that the way a phytoestrogen affects sperm quality depends on its type. Our findings show significant decreases of the weights of the left testicle and seminal vesicle in three of the administered dosages, but no significant difference in the weights of the left epididymis and prostate."

The results of the study show no significant differences of estradiol levels among the groups.

The administration of the olive extract in all of the three concentrations resulted in a significant decrease in both sperm count and sperm motility. All in all, it can be said that the way a phytoestrogen affects sperm quality depends on its type.

The authors findings show significant decreases of the weights of the left testicle and seminal vesicle in three of the administered dosages, but no significant difference in the weights of the left epididymis and prostate.

The authors opined that "The findings of the present study show that olive decreases the levels of reproductive indicators such as sperm count and motility, testosterone, the weights of testicle and seminal vesicle in male rats. The results of the study showed no change in the rats' weights; therefore, it can be concluded that the extract produces no effect on metabolism."

Based on this study the authors concluded, "olive fruit extract significantly decreased fertility parameters in the male adult rat. However, it is needed more study about the mechanism by which olive fruit extract creates its anti-fertility effects on human being which are still unknown. Nevertheless, considering our findings in this animal model, it is recommended that the olive fruit extract maybe used in the future as a contraceptive in males."

BioActor BV's comments

As this study involved olive fruit, it is difficult to draw a direct conclusion as to the results of this study and olive leaf extracts. It is generally accepted that extraction of whole fruit and other selected parts of a cultivar may lead to different compositions of the chemical components in the extract. Until that type of data becomes available, it is best not to speculate on any possible outcomes.

Additionally, the study was difficult to read and understand. It was not clear if the rats were randomized; the dosages of the olive fruit extract were not clear; the control group was not identified; and, the relevance of this study to humans was not clear.

2) **Ganjalikhan Hakemi S, Sharififar F, Haghpanah T, Babaee A, Eftekhar-Vaghefi SH. The Effects of Olive Leaf Extract on The Testis, Sperm Quality and Testicular Germ Cell Apoptosis in Male Rats Exposed to Busulfan. Int J Fertil Steril. 2019 Apr;13(1):57-65. doi: 10.22074/ijfs.2019.5520. Epub 2019 Jan 6. PMID: 30644246; PMCID: PMC6334023.**

This paper was intended to assess the effects of olive leaf extracts (OLE) as a source of antioxidants and phenolic compounds on Busulfan (BU)-induced damages in rat testes. Busulfan is used to treat a certain type of chronic myelogenous leukemia (CML; a type of cancer of the white blood cells). Busulfan is a class of medications called alkylating agents. It works by slowing or stopping the growth of cancer cells in your body.

Individuals taking BU may experience several side effects. Therefore, before taking this medication, individuals are advised of the following interactions and warnings:

"Interactions

Before taking this medication, tell your doctor if you've had:

- Blood/bone marrow disorders (such as bone marrow suppression, neutropenia, thrombocytopenia, anemia)
- Brain disorders (such as seizures, head injury)

Products that may interact with this drug include:

- Nalidixic acid

Warnings

- Pregnant women should avoid contact with this medication.
- Do not use if you are pregnant, suspect that you are pregnant, or while breastfeeding. Consult your doctor or pharmacist."

Studies have shown that BU has side effects on many organs such as the male reproductive system. The negative effects of BU on the male reproductive system include decreasing testis weight, increasing abnormal sperm parameters (motility and morphology), oligozoospermia, destroying almost all testicular germ cells, and causing temporary or permanent sterility.

Since BU is an alkylating agent with oxidative properties, it is believed that antioxidant therapy may be helpful in reducing its harmful effects.

This reasoning thus formed the basis for the experiments that are described below. Olive leaf is rich in antioxidant phenolic compounds such as oleuropein, verbascoside, ligstroside, as well as flavonoid compounds like tyrosol and hydroxytyrosol. Oleuropein scavenges harmful free radicals and prevents oxidative damage. It was reported that treatment with olive leaf extract (OLE) improved total antioxidant capacity (TAC) level in rat testicular tissue. Also, it was shown that OLE can improve sperm parameters and testis antioxidant conditions in rats exposed to rotenone.

In discussing the results of this study, the authors stated:

"The present study showed that administration of a single dose of BU to Wistar rats, leads to a significant reduction in sperm and testicular parameters (i.e., sperm viability and the number of PS and Leydig cells). Furthermore, our results demonstrated that BU could increase the rate of apoptotic SG and PS in the rat testis. However, it was shown that OLE administration at two doses of 250 and 500 mg/kg to rats that received BU, could significantly improve the afore-mentioned parameters in testis following BU-induced toxicity. Oral administration of OLE at 750 mg/kg has a negative effect in many cases (i.e., the thickness of germinal epithelium, spermatogenesis line- age cells, and apoptosis), and leads to increased levels of liver enzymes.

These findings are in line with previous reports showing toxic effects of BU in rat testis, including changes in sperm parameters and spermatogenesis along with pro-apoptotic BU potential in murine male germ cells. BU could induce oxidative damage to the testis. In addition, BU is an alkylating agent that by attaching to double strand DNA could prevent DNA replication and RNA transcription leading to stem cell death. These could explain inhibition of spermatogenesis process in the present study. In the present study, sperm motility decreased non- significantly in BU-exposed rats, and also sperm tail abnormality

was higher than those of the other groups. ROS can attack and damage bio-molecules such as DNA and lipids. As the sperm plasma membrane has a high content of polyunsaturated fatty acids, sperms are highly susceptible to oxidative stress. Oxidative stress induced by BU could affect the polyunsaturated fatty acids in the tail membrane of the sperm cell, disturb its fluidity and lead to a reduction in sperm motility. Also, previous studies showed that length of the sperm flagella reduces in rats that received BU, leading to decreased sperm motility.

In the current study, a decrease in the number of apoptotic germ cells was observed when OLE 250 and 500 mg/kg were administrated to Wistar rats treated with a single dose of BU.

In the present study, in line with toxic effects of OLE 750 mg/kg on the testis, an increase in liver enzyme levels in rats that received OLE 750 mg/kg, indicated liver damage (34) and suggested that this dose of OLE could be toxic. In addition, inflammation was observed in the liver of rats that received OLE 750 mg/kg. However, such changes are not seen following administration of other doses of OLE.

In the current study, no statistically significant difference was observed in liver enzymes levels between rats which received OLE 500 mg/kg for five weeks and the control and BU groups. However, some studies, in agreement with our data, showed that administration of olive extract at high doses may be associated with liver damage.

Previous studies demonstrated that the level of testosterone is different between control and BU groups (13). On the Contrary, in this study, there was no significant difference in total testosterone level among all groups. However, in this study, destruction rate of Leydig cells was higher in BU-treated than the control testes, while the number of Leydig cells increased significantly by all three doses OLE compared to BU-exposed testes. On the basis of our findings, we cannot attribute increased Leydig cell count in OLE-treated rats to the unchanged testosterone level."

The authors noted that further molecular and antioxidant studies are needed in order to determine the exact mechanism underlying the effects of different doses of OLE on BU-induced toxicity.

In conclusion, the authors stated: "This study, for the first time, showed that administration of two doses of OLE (250 and 500 mg/kg), to Wistar rats could improve BU-impaired spermatogenesis and sperm quality without inducing liver damage. However, OLE 750 mg/kg not only had no ameliorating effect on testis and sperm parameters in BU-exposed animals, but also increased apoptosis rate in the germ cell and enhanced liver enzymes that indicate a liver damage and probable dysfunctions of other important organs."

BioActor BV's comments:

The information in this paper shows mixed results. Some good, some bad. As the authors noted, additional research will be needed to address and verify these issues. In particular, the short duration of the studies does not allow one to consider any long-term effects, if any, of this treatment. Also, it would have been helpful if the paper had used an additional control absent BU, e.g., a control also using water which would help to isolate any individual effects of the treatment. Future studies to discern the relevance of this rat testes study to human testes is in order. It is presumed that individuals taking BU will be under the supervision and care of health professionals.

3) **Omer, Sawsan A., M. A. Elobeid, M. H. Elamin, Z. K. Hassan, P. Virk, M. H. Daghestani, E. M. Al-Olayan, Nadia A. Al-Eisa, and Z. M. Almarhoon. "Toxicity**

of olive leaves (Olea europaea L.) in Wistar albino rats." ***Asian J Anim Vet Adv*** **7, no. 11 (2012): 1175-82.**

In this study, Omer et al. evaluated the effects of olive leaf extracts on the hematology and biochemistry of the liver and kidney of Wistar albino rats fed an olive leaf extract for 6 weeks.

"Olive leaves were obtained from the Tabuk region of Saudia Arabia. The leaves were shade dried and were crushed to moderately coarse powder. A weight of 100 g of shade-dried leaves was ground in an electrical grinder and dissolved in 500 mL distilled water. The next day the mixture was strained out using fine sieve and the crude extract was air evaporated for three days. The concentrated OLE for the plant was then orally administered to rats by gastrogavage."

For this study, thirty, male Wistar white rats weighing 300-340 g were acclimatized to laboratory conditions for 7 days prior to the beginning of the experiment. The animals were then randomly assigned to 5 groups of 6 rats/group. Group 1 rats served as controls and were fed on the control diet. OLE extract was mixed with diet and administered by gastrogavage at concentrations of 0.2% (group 2), 0.4% (group 3), 0.7% (group 4) and 0.9% (group 5) every day for 6 weeks. The general health condition of the animals was monitored daily throughout the experimental period.

The authors reported the results of the study as follows.

"No clinical abnormalities were observed from the experimental animals throughout the course of the experiment. Animals did not show any signs of sickness and there was no obvious clinical manifestation like weakness, diarrhea or yellow discoloration of the mucus membranes."

" … There was significant decrease in the RBCs, PCV, MCHC in group 5 compared to the control group ($p<0.05$). The other groups did not show significant differences in the erythrocytic series. However, group 2 showed significant decrease in both the HB and the PCV compared to the control group ($p<0.05$). The MCV was significantly higher in groups 3, 4 and 5. The WBCs showed marked reduction in the test groups compared to the control group."

"Serum concentrations of Alkaline Phosphatase (ALP), Lactate Dehydrogenase (LDH), Total Bilirubin (TBil) and cholesterol, glucose, triglycerides as well as hematological profiles were determined in the present study for each group of rats. There was a significant increase in the serum levels of ALP and total bilirubin in groups 3 and 4 and 5 compared to the control group. There was also significant decrease in the serum triglyceride, glucose and cholesterol in test groups as compared to the control group. The haematological profile showed significant decrease in the values of red blood cell counts, haemoglobin and packed cell volume of the animals in group 5. Microscopically both liver and kidneys showed histological alterations in the form of fatty cytoplasmic vacuolation, necrosis of the hepatocytes and a slight hemorrhage was recorded in the kidneys of the experimental animals especially those fed 0.9% olive leaf extract."

The authors stated their conclusion below:

Feeding olive leaves extracts to Wistar albino rats at a dose up to 0.9% resulted in hepatocellular and renal abnormalities. Olive leaves extracts in the present study, however, has resulted in lowering cholesterol and blood glucose. Therefore, further work is needed to determine the correct dose, to be used over long time which results in minimal or no pathological changes and at the same time continue to reducing blood sugar and cholesterol."

BioActor BV's comments

This study leaves us wondering if the study could be replicated by other scientists in that there does not appear to be enough information provided to allow other scientists to exactly duplicate the experiment as described. The olive leaf extract was not adequately characterized to enable other researchers to define the material and compare it to other similar extracts. The weight of the extract used was not mentioned. We do agree with the authors that additional research is needed in this area.

b. Over all summary

In summary, the adverse effects noted in the above described articles are difficult to explain. All these publications appeared in not so well known journals, with several draw backs such as, the studies are not well conducted, the extract is not well characterized, etc. In several other rat studies with olive product, the possibility of such adverse effects was not indicated. It is unlikely that the effects noted in the above studies were species-specific. These effects, if true, may be triggered by some other mechanisms, but this remains to be investigated. Contrary to the above reported adverse effects, the available evidence from historical and current uses of olive and its products as well significant number of studies in animals and humans support the safety of olive and its products, including leaf extract.

The leaves of olive trees have also been consumed traditionally for health purposes. The use of olive leaf use as a febrifuge has been cited in nineteenth century references (Hanbury, 1854, Pallas, 1828). Various olive leaf extract products are currently sold in the marketplace. Recently, interest in the high polyphenolic levels in olive leaves has led to the study of enhancing olive and other edible oils with olive leaf extracts to increase phenolic concentrations which resist oxidative deterioration (Bouaziz et al., 2008; Japon-Lujan and Castro, 2008).

Polyphenols as components of olive oil or olive leaf extract have been investigated for their potential health effects in multiple clinical studies. In several published review articles (Raederstorff, 2009; EFSA, 2011; Rigacci and Stefani, 2016; Tsartsou et al., 2019), the available human clinical studies of olive polyphenols have been summarized. A majority of the clinical studies of olive polyphenols are conducted to evaluate the efficacy. These intervention studies suggest that olive polyphenols protects against oxidative damage. The available information suggests olive leaf extract is unlikely to cause adverse effects.

As described in the GRAS notice, there is sufficient qualitative and quantitative scientific evidence, including animal data, to assess the safety-in-use for olive leaf extract. The safety assessment of olive leaf extract is based on the totality of available evidence, including specifically designed animal toxicity studies. The totality of the available evidence supports the safety of olive leaf extract at the proposed use levels and resulting in maximum (90^{th} percentile) intake of 447.5 mg/day. The intended uses are compatible with current regulations, i.e., olive leaf extract is used in specified foods (described in the GRAS notice) and is produced according to current good manufacturing practices (cGMP).

Bouaziz M, Fki I, Jemai H, Ayadi M, Sayadi S., 2008. Effect of storage on refined and husk olive oils composition: Stabilization by addition of natural antioxidants from Chemlali olive leaves. Food chemistry. 108(1):253262.

EFSA. 2011. European Food Safety Authority. Panel on Dietetic Products, Nutrition and Allergies (NOA); Scientific Opinion on the substantiation of health claims related to polyphenols in olive and protection. EFSA J. 9(4):2033 [25pp.].

Hanbury D. 1854. On the febrifuge properties of the olive (*Olea europaea*, L.). Pharmaceutical Journal and Transactions. XIII:353-354.

Japon-Lujan R, Luque de Castro MD., 2008. Liquid-liquid extraction for the enrichment of edible oils with phenols from olive leaf extracts. J Agric Food Chem. 56(7):2505-2511.

Pallas E. 1828. Analyse chimique des feuilles et des ecorces d'olivier, suivie d'observations medicales sur l'emploi de pextrait obtenu de l'ecorce du meme vegetal dans le traitement des fievres intermittentes. Journal Universel des Sciences Medicales. 1828:257-299.

Raederstorff, D. 2009. Antioxidant activity of olive polyphenols in humans: A review. Int. J. Vitam. Nutr. Res. 79(3):152-165.

Rigacci, S., Stefani, M., 2016. Nutraceutical properties of olive oil polyphenols. An itinerary from cultured cells through animal models to humans. International J. Mole. Sci. 17(6): 843.

Tsartsou, E., Proutsos, N., Castanas, E., Kampa, M., 2019. Network meta-analysis of metabolic effects of olive-oil in humans shows the importance of olive oil consumption with moderate polyphenol levels as part of the Mediterranean diet. Front. Nutr. 6:6. doi: 10.3389/fnut.2019.00006.

8. **Because Clewell et al. conducted a battery of toxicological studies using water-soluble extract of olive leaves providing a pivotal publication to support the safety of the proposed GRAS ingredient, please confirm whether the test material used in these studies was the same as the article of commerce, particularly in terms of the polyphenol composition. Also, please provide the accurate citation of the Clewell et al. study.**

BioActor BV confirms that the test material, olive leaf extract, used in the battery of toxicological studies with olive leaf extract (the subject of GRAS notification) was the same as the article of commerce, particularly in terms of the polyphenol composition.

The accurate citation for the Clewell et al. (2016) study is:

Clewell AE, Béres E, Vértesi A, Glávits R, Hirka G, Endres JR, Murbach TS, Szakonyiné IP., 2016. A Comprehensive Toxicological Safety Assessment of an Extract of Olea Europaea L. Leaves (Bonolive™). Int J Toxicol. 35(2):208-21. doi: 10.1177/1091581815619764.

From:	Hans van der Saag
To:	Downey, Jason
Cc:	Madhu Soni; Eleonora Panaro
Subject:	[EXTERNAL] Re: GRN 001119 - BioActor"s Olive Leaf Extract - Answers to Questions to the Notifier
Date:	Wednesday, September 27, 2023 9:30:20 AM

Hi Jason,

Good talking with you and Diana!

As it happens, I did send the mail, but did not include you as recipient. Our answers you find below:

I acknowledge receipt of your mail of September 20, last below.

Please find below our answers to your additional questions:

Question 1. It is noted that there is variability of bioactive compounds in natural products. Factors that contribute to this variability include a.o. the type of cultivar, geographic location, season, climate, harvesting time, methods of cultivation, post-harvest drying and storage conditions [Ref.: Medina et al., 2019]. With respect to olive leaves, oleuropein is the major phenol in olive leaf extracts. Given the variability of the content of oleuropein in different lots, it may from time to time be necessary to co-blend different lots to meet the specifications of oleuropein levels in the commercial product. For example, if one lot is lower than the specified content for oleuropein and another lot is higher in oleuropein content, these lots are blended to meet the required specifications. We understand that this is a common practice in natural products.

Reference: Medina E , Romero C , García P , Brenes M., 2019. Characterization of bioactive compounds in commercial olive leaf extracts, and olive leaves and their infusions. Food Funct. 10(8):4716-4724. doi: 10.1039/c9fo00698b.

Question 2. We confirm that we have adjusted our specifications for heavy metals to reflect the results of our batch analyses. The values are now as follows:

lead - 0.5 ppm
cadmium - 0.5 ppm
mercury - 0.1 ppm
arsenic - 0.5 ppm

With respect to the arsenic levels in olive leaf extracts, we note that the arsenic content can also vary due to factors as commented in our answer to question 1. While the arsenic level for one lot is much higher than the arsenic levels for other lots, we note that this arsenic level is still much lower than the arsenic specification level of 1 ppm. Given this, we will continue to monitor the arsenic level in our olive leaf extract lots, and if the arsenic level is higher than the established specifications, that lot will be discarded. Please note that we have revised the arsenic specification to 0.5 ppm.

Please confirm receipt of this mail,

Thanks and regards

Hans

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From: Downey, Jason <Jason.Downey@fda.hhs.gov>
Date: Wednesday, 20 September 2023 at 19:42
To: Hans van der Saag <hans.vandersaag@bioactor.com>
Subject: GRN 001119 - BioActor's Olive Leaf Extract - Questions to the Notifier

Hi Hans,

During our evaluation of GRN 001119 and your August 28, 2023, amendment, we noted additional questions and points in need of clarification, which are listed below. Please provide responses to these requests within 10 business days. If you foresee any issue with this timeline or you have any other questions, please contact me as soon as possible.

Thank you in advance for your attention to our comments.

Sincerely,

Jason

Jason Downey, Ph.D. (he/him/his)
Regulatory Review Scientist

Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
jason.downey@fda.hhs.gov

FDA Questions to the Notifier

1. In response to FDA's question 2 of our July 20, 2023 email, you provided a more detailed narrative of the manufacturing process for olive leaf extract. In your August 28, 2023, response, you stated that "before the final packaging step in polyethylene bags, the spray dried extract is milled and standardized". Please describe how the ingredient is standardized to ensure that the ingredient meets the specifications.

2. In question 2.e. of our July 20, 2023, email, we asked you to provide the actual values for the analyses of 4 batches of olive leaf extract. We also requested that your specifications for heavy metals be as low as possible and representative of the results from your batch analyses to align with FDA's Closer to Zero initiative that focuses on reducing the dietary exposure to heavy metals. We note that in your August 28, 2023, response, the specifications for heavy metals remained unchanged from the original specifications provided in the notice and have not been adjusted to reflect the results of your batch analyses. In addition, we note that the arsenic level for batch no. PF0348220321 is considerably higher than the other batches (i.e., 0.38 mg/kg versus <0.020 mg/kg). Please revise your specifications to be as low as possible based on the results of your batch analyses and address the variability in the arsenic results.

From:	Hans van der Saag
To:	Downey, Jason
Cc:	Patricia Dias
Subject:	Re: [EXTERNAL] Re: GRN 001119 (BioActor"s olive leaf extract) update - Intended Uses + Heavy Metal Levels
Date:	Tuesday, November 28, 2023 12:17:18 PM
Attachments:	image001.png image002.png Updated Table 7 & Annex A (28-11-2023).pdf

Hi Jason

This mail answers the queries you raised with regards to the Intended Uses and the Heavy Metals Levels

1. **Intended Uses**

We had to go back to the draft submission that was prepared in 2017 with the original list of Intended Uses and the original Annex A. We replaced table 7 and Annex A with the versions of 2017 that are based on the Crème analysis for the exposure estimate; see the attachment as well as the table below:

Updated Table 1 & . Bonolive® Intended Uses*

Food Category	Maximum Use (ppm)
Yogurts	1111 ppm
Flavored Milk Drinks	1042 ppm
Dry Powdered Milk and Milk Mixtures (Not Reconstituted)	8333 ppm
Coconut Beverages	1042 ppm
Cookies (Certain Categories*)	8333 ppm
Cereal, Granola and Nutrition Bars	8333 ppm
Cereals (Certain Categories*)	8333 ppm
Plums (Dried/Fresh)	6250 ppm
Fruit Juices and Nectars (Including Citrus)	1042 ppm
Vegetables and Vegetable Juices (e.g. Carrot and Tomato Juice)	1042 ppm
Fruit-Flavored Beverages (Ready to Drink and from Powders)	1042 ppm
Vegetable and Fruit Juice Blends	1042 ppm
Fortified Water	1042 ppm
Carbonated Soft Drinks	1042 ppm
Teas	1042 ppm
Nutrition Drinks and Powders	1042 ppm
Sports Drinks	1042 ppm
Table Fats (Certain Categories*) and Vegetable Oils (Olive oil)	16667 ppm
Candies (Dark Chocolate**, Gum Drops, Hard Candy, Dietetic Candy)	8333 ppm
Chewing Gum (Sugar Free)	83333 ppm

*See Appendix A for a full list of food categories

** The intended use will not include foods in which a standard of identity precludes its use.

Furthermore, please find the answers to your questions below:

- Carbonated beverages – This category was included in the dietary exposure estimate but was not included in the list of intended uses. If this is an intended use, please revise the table to include this food category and the corresponding use level in this category. If this is not a food category in which the ingredient is intended to be used, please revise the dietary exposure accordingly. We have included carbonated beverages in Table 7, as it is an intended use that is also comprised in Annex A and used for the dietary exposure estimate.

- Cookies – In the amendment, you provided a list of cookies that were excluded from the dietary exposure estimate. However, we note that these cookies were previously included in the dietary exposure estimate. Please clarify in which cookies the ingredient is intended to be used and ensure that the dietary exposure estimate is reflective of those intended uses. We have included the original list of cookies in Annex A and updated table 7 with a reference to Annex A, as they are used in the dietary exposure estimate.

- Vegetable juices – Only food codes for tomato juice were included in the dietary exposure estimate. Please indicate if the intended use is limited to tomato juice only and not other vegetable juices. The intended use is not limited to tomato juice, but also comprises the food codes for carrot juice and other vegetables (excluding tomato). Codes 73105010, 75132000

- Table fats – Only food codes for margarine and vegetable spreads were included in the dietary exposure estimate. Please clarify if the intended use is only in margarine and vegetable spreads or if other table fats, such as salad dressings, are included as intended uses for this food category. Yes, the intended use is only of Margarine and Vegetable spreads. See updated Table 7 in which we clarify:" Table Fats (Certain Categories*)"

- Vegetable oils – Only the food code for olive oil was included for this food category. Please clarify if the ingredient is only intended to be used in olive oil and not all vegetable oils. Yes, the intended use is only Olive oil. See updated Table 7 whereby we clarified: " Vegetable Oils (Olive oil)"

- Candies – Please indicate if the intended use is in only in hard candy and gumdrops, as well as chocolate. We note that "chocolate" has a standard of identity that may preclude the use of certain ingredients. Please indicate that the intended uses will not include foods in which a standard of identity precludes its use. We updated the List of Intended Use, by adding to dark chocolate candies the statement "** The intended use will not include foods in which a standard of identity precludes its use.".

- Teas and coffee – No food codes for coffee were included in the dietary exposure estimate. Please indicate if coffee is a food category in which the ingredient is intended to be used. You are right, our exposure estimate was calculated without coffee as an intended use. We took out 'coffee' from Table 7, which is now in sync with Annex A.

- Chewing gum – Please indicate if the intended use is in all chewing gum or if the intended use is limited to sugar free gum. We note that only one food code for chewing gum was included in the dietary exposure estimate. Yes, the intended use is only for Sugar Free Chewing Gum and the exposure estimate was made on the basis of that assumption. See updated Table 7 whereby we clarified: " Chewing Gum (Sugar Free))"

2. **Heavy Metals Levels**

We took a closer look to our CoA's results and propose the following maximum levels:

Lead:	0.1	ppm
Cadmium:	0.05	ppm
Mercury:	0.05	ppm
Arsenic:	0.35	ppm

Regards,
Hans

Hans van der Saag
CEO & Founder

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From: Downey, Jason <Jason.Downey@fda.hhs.gov>
Date: Tuesday, 28 November 2023 at 13:43
To: Hans van der Saag <hans.vandersaag@bioactor.com>
Subject: RE: [EXTERNAL] Re: GRN 001119 (BioActor's olive leaf extract) update

Thanks, Hans! I appreciate the update.

From: Hans van der Saag <hans.vandersaag@bioactor.com>
Sent: Monday, November 27, 2023 6:13 PM
To: Downey, Jason <Jason.Downey@fda.hhs.gov>
Subject: [EXTERNAL] Re: GRN 001119 (BioActor's olive leaf extract) update

Hi Jason,

First of all, my apologies for the radio silence; I'm finishing our response by Tuesday Nov 28th.

No further delays or excuses,

Regards
Hans

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From: Downey, Jason <Jason.Downey@fda.hhs.gov>
Date: Monday, 27 November 2023 at 14:09
To: Hans van der Saag <hans.vandersaag@bioactor.com>
Subject: GRN 001119 (BioActor's olive leaf extract) update

Hi Hans,

I wanted to check in with you because I have not yet received BioActor's responses to FDA's questions/requests from September 28 and October 17 about your GRAS notice on olive leaf extract. Also, I did not receive a response to my November 20 email to you asking for an update. As there is little time left for us to respond to your GRAS notice, we will have to begin drafting a response letter stating your GRAS notice does not provide a basis to conclude your intended uses of olive leaf extract are GRAS if your notice is not completed soon.

Please let me know on what date we can expect BioActor's responses to our questions/requests.

Thank you!

Jason

Jason Downey, Ph.D. (he/him/his)
Regulatory Review Scientist

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From: Hans van der Saag
To: Downey, Jason
Cc: Patricia Dias; Madhu Soni
Subject: [EXTERNAL] Re: GRN 001119 - Question to the Notifier
Date: Monday, January 29, 2024 11:29:11 AM

Hi Jason,

Reference is made to your mail of Jan 16th, last.

I hereby confirm that:

- the food codes for Cereals (Certain Categories) listed in the amended Annex A; and
- the food codes for Plums (Dried/Fresh) listed in the amended Annex A

were included in the dietary exposure estimate provided in GRN 001119 even though they were not included in Annex A in the original submission.

Best regards

Hans van der Saag
CEO BioActor

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From: Downey, Jason <Jason.Downey@fda.hhs.gov>
Date: Tuesday, 16 January 2024 at 13:45
To: Hans van der Saag <hans.vandersaag@bioactor.com>
Subject: GRN 001119 - Question to the Notifier

Hi Hans,

During our evaluation of GRN 001119 and its amendments, we noted a point in the dietary exposure section that needs clarification:

In the amendment dated November 28, 2023, you indicated that you reverted to an original list of intended uses from 2017 and provided an updated table of the intended uses (Table 1 and 7) and food codes (Annex A) used in the dietary exposure estimate. We note that food categories for certain cereals and dried and fresh plums were added to the revised intended use table in the amendment. Please clarify if these food codes were included in the dietary exposure estimate

provided in GRN 001119 even though they were not included in Annex A in the original submission. If these food codes were not included in the dietary exposure estimate, please remove these intended uses or revise the dietary exposure estimate to include these food categories.

Please provide the requested clarification within 10 business days. If you foresee any issue with the timeline or you have any other questions, please contact me as soon as possible. It would be helpful for me if you confirmed receipt of this email.

Thank you!

Jason

Jason Downey, Ph.D. (he/him/his)
Regulatory Review Scientist

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From: Hans van der Saag
To: Downey, Jason
Subject: [EXTERNAL] Re: GRN 001119 administrative question
Date: Tuesday, January 30, 2024 9:20:18 AM
Attachments: image001.png
image002.png

Hi Jason,

I double-checked the reference list (section 7.2 of the submission) and the latest reference (nr. 101) is from **June 2022**, Other references from 2022 are :38, 76 and 90.

Best regards
Hans

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CEO BioActor

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From: Downey, Jason <Jason.Downey@fda.hhs.gov>
Date: Tuesday, 30 January 2024 at 14:45
To: Hans van der Saag <hans.vandersaag@bioactor.com>
Subject: GRN 001119 administrative question

Hi Hans,
We have a quick question to clarify the administrative record. Can you confirm through what month and year the literature search was conducted for GRN 001119?
Thank you!
Jason

Jason Downey, Ph.D. (he/him/his)
Regulatory Review Scientist

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