NEW DIETARY INGREDIENT (NDI) SAFETY INFORMATION

OLIVE DRY EXTRACT TITRATED IN HYDROXYTYROSOL – OLEANOX

Table of Contents

Table of Figures

Table of Tables

List of Annexes

Annex 1_ Certification Innovaoleo confidential
Annex 2_ Certification Natac confidential
Annex 3_ Specification 10% extract
Annex 4_ Specification 20% extract
Annex 5_ Heavy metals analysis confidential
Annex 6_ Microbiology analysis confidential
Annex 7_ Mycotoxins analysis confidential
Annex 8_ PAH analysis confidential
Annex 9_ PCB dioxins analysis confidential
Annex 10_ Pesticides analysis confidential
Annex 11_ Eurofins accreditation
Annex 12_ Phytolab accreditation
Annex 13_ Nutritional analysis confidential
Annex 14_ CoA 10% extract confidential
Annex 15_ CoA 20% extract confidential
Annex 16_ HPLC method validation confidential
Annex 17_ CIDAF certification
Annex 18_ HPLC analysis confidential
Annex 19_ Stability study report confidential
Annex 20_ CoA HT-10 and 20 Stability baseline confidential
Annex 21_ CoA HT-10 accelerated confidential
Annex 22_ CoA HT-20 accelerated confidential
Annex 23_ CoA HT-10 intermediate confidential
Annex 24_ CoA HT-20 intermediate confidential
Annex 25_ CoA HT-10 long-term confidential
Annex 26_ CoA HT-20 long-term confidential
Annex 27_ USA olive consumption Datamonitor
Annex 28_ Bacterial reverse mutation test report confidential
Annex 29_ In vitro micronucleus test report confidential
Annex 30_ Allergen statement 10% extract confidential
Annex 31_ Allergen statement 20% extract confidential

List of Abbreviations

°C	degrees Celsius
µm	micrometers
ADME	Absorption Distribution Metabolism and Excretion
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BV	bed volume
BW	body weight
CAS	Chemical Abstracts Service
CFU	colony forming unit
CP	control point
CRP	c-reactive protein
E. coli	*Escherichia coli*
EFSA	European Food Safety Authority
FDA	Food and Drug Administration
g	grams
γ-GT	gamma-glutamyltranspeptidase
GC-MS	gas-chromatography-mass spectrometry
GRAS	generally recognized as safe
GRN	GRAS number
ha	hectare
HACCP	hazard analysis critical control point
HbA1c	glycohemoglobin, hemoglobin, A1C
HDL	high density lipoprotein
HPLC-ESI-QTOF-MS/MS	high performance liquid chromatography-diode array detection-electrospray ionization-quadrupole time-of-flight-mass spectrometry
HPLC-FLD	high-performance liquid chromatography with fluorescence detection
IC-PAD	ion chromatography with pulsed amperometric detection
IUPAC	International Union of Pure and Applied Chemistry
kg	kilograms
LD_{50}	median lethal dose
LDL	low density lipoprotein
LDPE-LLDPE	low density polyethylen-linear low-density polyethylene
LOD	limit of detection
LOQ	limit of quantification
MS/MS	tandem mass spectrometry
MTD	maximum tolerated dose
NDI	new dietary ingredient
NDIN	new dietary ingredient notifications
NOAEL	no observable adverse effect level
O. europaea	*Olea europaea*
ODI	old dietary ingredient
OECD	Organisation for Economic Co-operation and Development

PAH	polycyclic aromatic hydrocarbons
PCB/TEQ	polychlorinated biphenyls/toxic equivalents
PCDD/F+PCB TEQ	polychlorinated dibenzo-p-dioxins and polychlorinated dibenzo dibenzofurans/polychlorinated biphenyls/toxic equivalents
PCDD/F-TEQ	polychlorinated dibenzo-p-dioxins and polychlorinated dibenzo dibenzofurans/toxic equivalents
ppb	part per billion
ppm	part per million
RT	Retention time
S. aureus	*Staphylococcus aureus*
S. typhimurium	*Salmonella typhimurium*
TAMC	total aerobic microbial count
TYMC	total combined yeasts and molds count
US	United States
UV	ultra violet

1. New Dietary Ingredient Identity Information (Recommended)

1.1 Description of the identity of the NDI

The current NDI consists in *Olea europaea* L. fruit extract, standardized to either 10% minimum hydroxytyrosol or 20% hydroxytyrosol. The NDI brand name is OLEANOX.

According to theplantlist.org, the full name of olive is *Olea europaea.* The taxonomy of olive is:

Kingdom: Plantae
Subkingdom: Virideplantae
Division: Tracheophyta
Subdivision: Spermatophytina
Superorder: Asteranae
Order: Lamiales
Family: Oleaceae
Genus: Olea
Species: *Olea europaea* L.

Still according to theplantlist.org, *O. europaea* has many synonyms*:*
Olea alba Lam. ex Steud., *Olea amygdalina* Gouan, *Olea angulosa* Gouan, *Olea argentata* Clemente ex Steud., *Olea atrorubens* Gouan, *Olea bifera* Raf., *Olea brevifolia* Raf., *Olea cajetana* Petagna, *Olea cayana* Raf., *Olea craniomorpha* Gouan, *Olea europaea* var. *buxifolia* Aiton, *Olea europaea* var. *communis* Aiton, *Olea europaea* subsp. *europaea*, *Olea europaea* var. *ferruginea* Aiton, *Olea europaea* var. *latifolia* Aiton, *Olea europaea* var. *longifolia* Aiton, *Olea europaea* var. *obliqua* Aiton, *Olea europaea* subsp. *oleaster* (Hoffmanns. & Link) Negodi, *Olea europaea* var. *sativa* (Weston) Lehr, *Olea europaea* subsp. *sativa* (Weston) Arcangeli, *Olea europaea* var. *sylvestris* (Mill.) Lehr, *Olea europaea* subsp. *sylvestris* (Mill.) Hegi, *Olea europaea* var. *sylvestris* (Mill.) Lehr., *Olea ferruginea* (Aiton) Steud., *Olea gallica* Mill., *Olea hispanica* Mill., *Olea lancifolia* Moench, *Olea longifolia* (Aiton) Steud., *Olea lorentii* Hochst., *Olea obliqua* (Aiton) Steud., *Olea oblonga* Gouan, *Olea odorata* Rozier ex Roem. & Schult., *Olea officinarum* Crantz, *Olea oleaster* Hoffmanns. & Link, *Olea polymorpha* Risso ex Schult., *Olea praecox* Gouan, *Olea racemosa* Gouan, *Olea regia* Rozier ex Roem. & Schult., *Olea sativa* Weston, *Olea sphaerica* Gouan, *Olea sylvestris* Mill., *Olea variegata* Gouan, *Olea viridula* Gouan, *Phillyrea lorentii* Walp.

OLEANOX is standardized in hydroxytyrosol (10 or 20%). Hydroxytyrosol (3,4-dihydrophenylethanol) has a molecular weight of 154.16 g/mol and its molecular formula is $C_8H_{10}O_3$. Its IUPAC name is 4-(2-hydroxyethyl)benzene-1,2-diol. Its CAS number is 10597-60-1. The structure of hydroxytyrosol is presented below.

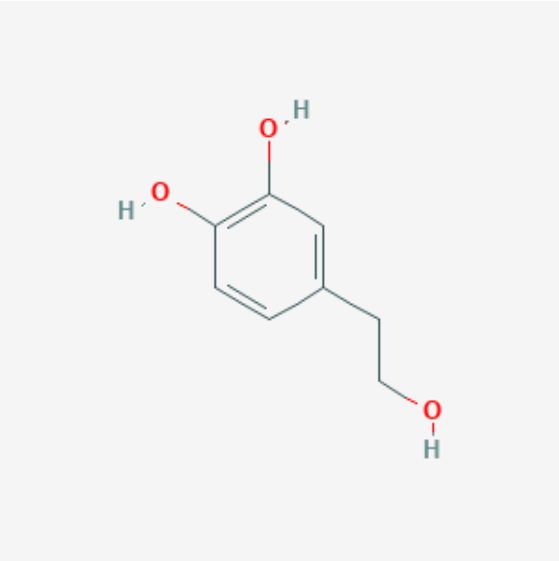


Figure 1: Structure of hydroxytyrosol.

The fruit of olive tree and olive oil are consumed worldwide. The Food and Agriculture Organization has estimated that that the worldwide production of olives was 19,267,000 tons in 2016, with Spain, Greece and Italy as the main producers. Olive oil consumption is also important, with for instance an estimated consumption over 24 liters per person per year in Greece, 14 liter per person per year in Italy and Spain. Outside Europe, olive oil is also consumed, with estimation around 8 liter per person per year in Tunisia, Portugal, Syria, Jordan and Lebanon. Consumption in UK, USA or Canada is lower, around 1 to 1.5 liter/person/year.

In 2009, olive oil consumption was quite similar, and the world consumption has been estimated to be in average 0.43 liter/year/person, with Greece (23.7 L/year/person), Spain (13.62 L/year/person) and Italy (12.35 L/year/person) being the three highest consumers of olive oil (Ghanbari *et al.,* 2012).

In the US, *Olea europaea* L. is listed as ODI (old dietary ingredient), *i.e*. ingredients sold on the US market in dietary supplements before 15 October 1994. Moreover, a New Dietary Ingredient notification (NDI 351) has been submitted in 2006 for an olive fruit extract (100 mg/day, equivalent to 35 mg/day polyphenols, particularly 5 mg/day hydroxytyrosol and 0.3 mg/day tyrosol). FDA had objection on this notification due to the lack of manufacturing process and of a specific method of analysis of polyphenols.

Still in the USA, a GRAS application (No 726) has been submitted for an olive fruit extracts, standardized at ≥ 40% hydroxytyrosol. The extract can be used in 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, at a level of 5 to 10 mg of hydroxytyrosol per serving.

Regarding hydroxytyrosol, two GRAS notices have been submitted (No 600 and 876), one related to hydroxytyrosol produced by chemical synthesis (No 600) and the second one produced by fermentation of a culture of *E. coli* (No 876). These two applications were related to the use of hydroxytyrosol as additive. GRN 876 intended to use hydroxytyrosol as an antioxidant in bakery products, beverages, dairy products and substitutes, desserts, fats and oils, fruits 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 to 10 mg hydroxytyrosol per serving. Regarding the GRN 600 notice, hydroxytyrosol was intended for use as an antioxidant and antimicrobial in beverages, fats and oils, fresh and processed fruits and vegetables and juices, and gravy and sauces at use levels of 5.0 milligrams per serving.

NATAC Biotech S.L. intends for OLEANOX to be used in dietary supplements under the following conditions:

- Users of the dietary supplement should be healthy adults. Should not be used by children, during pregnancy or lactation.
- Avoid using the dietary supplement and/or consult with a physician if you have a medical condition or taking prescription medications.
- The maximum recommended daily dose of the NDI is 20 mg of hydroxytyrosol, which corresponds to 200 mg of the 10% extract or 100 mg of the 20% extract. Manufacturers of finished dietary supplements may choose to deliver this amount in a single serving or divide this total into correspondingly reduced serving amounts taken twice a day or three time a day.

Based on FDA's draft guidance for industry entitled "Dietary Supplements: New Dietary Ingredient Notifications [NDINs] and Related Issues" (August 2016) (FDA 2016), the intake pattern should be considered to be "intermittent." Per FDA 2016 (page 70): "Intermittent use, for purposes of this guidance, means less than daily chronic use and can be either daily and finite in duration or non-daily and lifetime in duration."

1.2 Description of the evidence verifying the identity of the NDI (Trade secret)

(b) (4) [redacted] Olive can be easily identified based on the plant source, its color, odor, taste and appearance. Additionally, the product identity and quality are standardized by parameters such identification of hydroxytyrosol by HPLC and levels of hydroxytyrosol (10 to 20%).

1.3 NDI manufacture (Trade secret)

1.3.1 Raw materials (Trade secret)

(b) (4) [redacted]

The variability from batch to batch in term of composition of the olive juice was evaluated by HPLC and is presented in the figure below. No variability from batch to batch of olive juice composition was noted.

(b) (4)

Figure 2: Variability in olive juice composition from different olive fruit batches by HPLC.

1.3.2 Formulation ingredients

Not applicable.

1.3.3 Manufacturing process (Trade secret)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

Figure 3: Flow-chart of olive oil extraction and production of olive juice

(b) (4)

(b) (4)

(b) (4)

Figure 4: Elution of dry matter (in blue) and hydroxytyrosol (in red) by size-exclusion chromatography

(b) (4)

(b) (4)

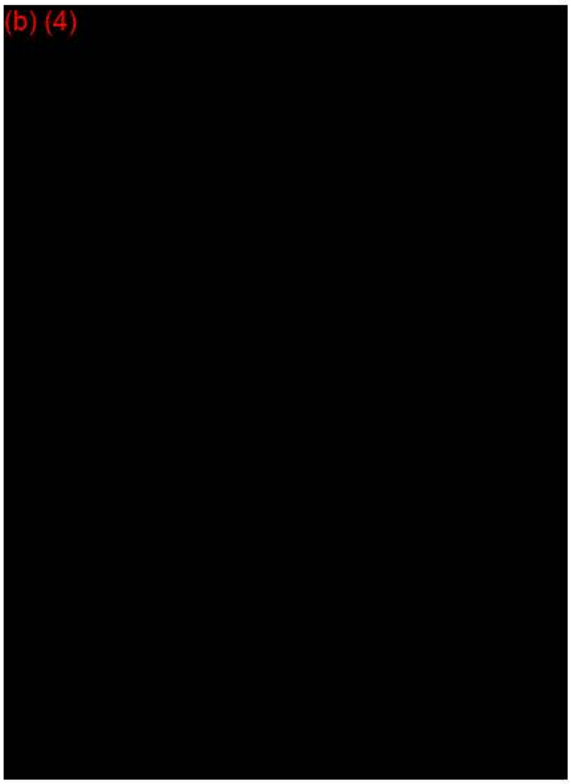


Figure 5: Flow-chart of OLEANOX

Certifications FSSC 22000 (Food Safety System certification) are available for:

- Innovaoleo, S.L. (b) (4) (***Annex 1***)
- Natac Biotech S.L. (b) (4) (***Annex 2***).

Controls points (see *Figure 5*):

Control point 1 (CP1):

(b) (4)

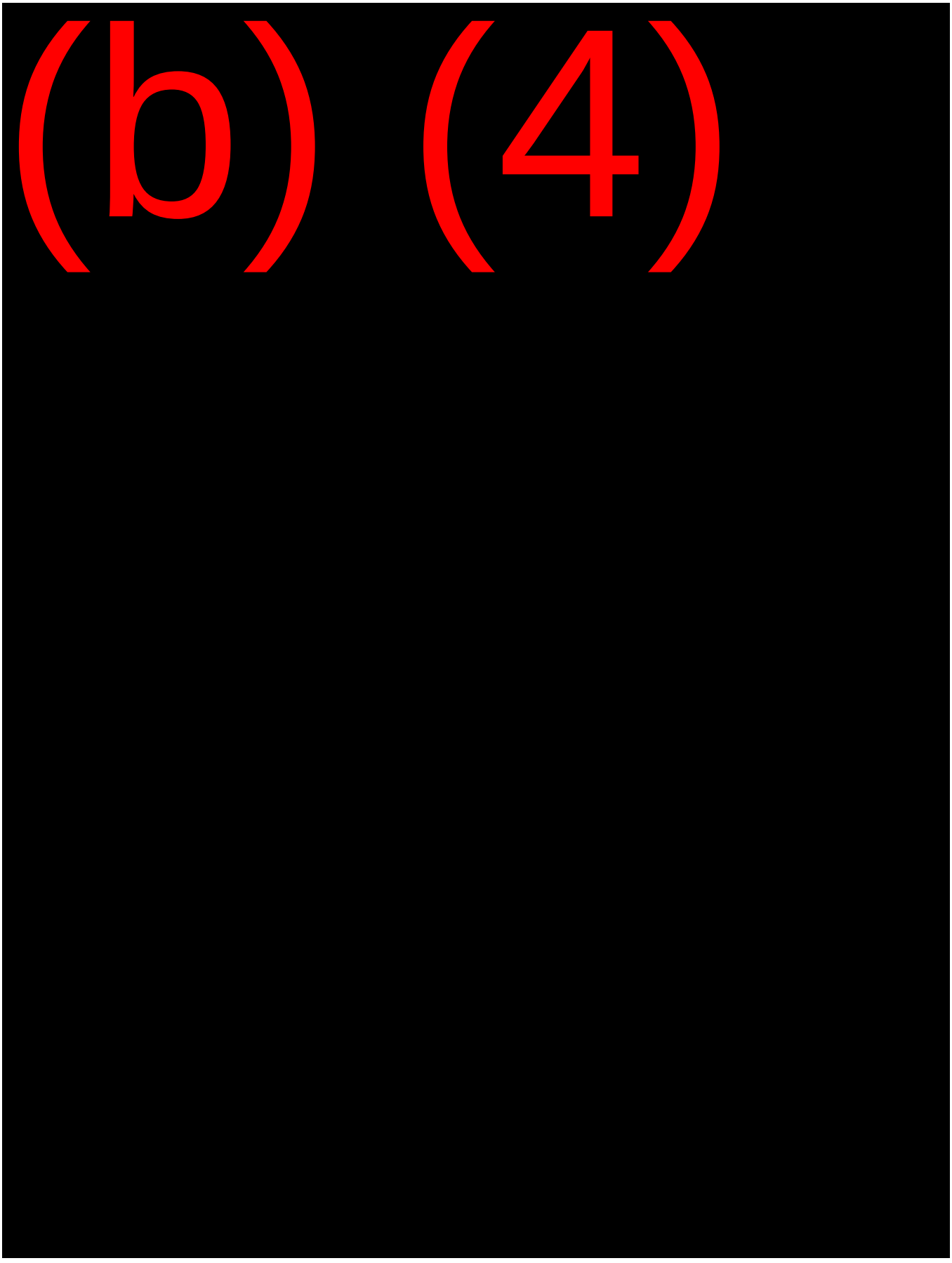
Table 1: Required values of hydroxytyrosol in both extracts after purification (control point).

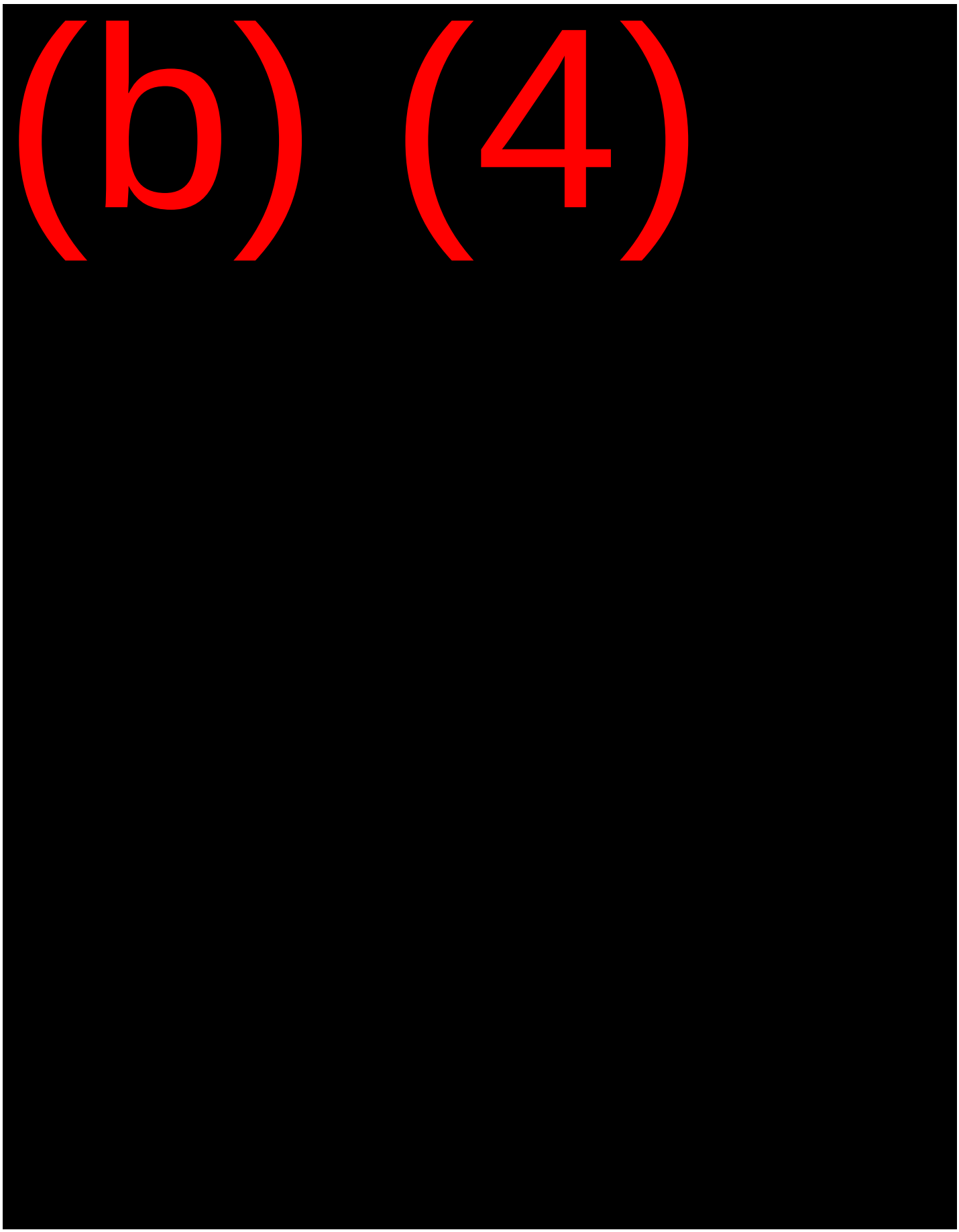
(b) (4)

Control point 2 (CP2):

(b) (4)

1.3.4 NDI specifications




(b) (4)

All analyzes have been conducted by Eurofins or Phytolab. The accreditations of the laboratory are presented in ***Annex 11*** and ***Annex 12***. (b) (4)

Table 2: Analysis of the contaminants in three batches of each extract.

(b) (4)


(b) (4)

In addition, a nutritional analysis for both the 10% and 20% extracts (three batches per extract) are presented in ***Annex 13*** and in the next table.

(b) (4) [redacted].

Table 3 : Nutritional analysis of three batches of each extract.

(b) (4)

1.3.5 Methods of analysis (Trade secret)

OLEANOX is standardized to either 10% or 20% hydroxytyrosol. Hydroxytyrosol has been analyzed in three batches of each product by using HPLC method (Annex ***14*** and Annex ***15***).

Table 4: Results of hydroxytyrosol analysis in various batches of the extract.

(b) (4)

[redacted]

(b) (4) [redacted] This method and its validation are described in ***Annex 16***. The table below summarizes all the results obtained from this validation study, to be compared with the acceptance criteria.

Table 5: Validation of the (b) [redacted] ***method for the assessment of hydroxytyrosol and tyrosol.***

(b) (4)

[redacted]

As shown in this table, all the parameters comply with acceptance criteria, which allows to validate the method for the analysis of hydroxytyrosol and tyrosol in olive extracts standardized in hydroxytyrosol.

In Addition to this internal evaluation, the analysis of the two extracts (10 and 20%) was performed by a third-part laboratory (CIDAF). CIDAF is certified to ISO 9001:2015, ISO 14001:2015 and UNE 166002:2021 ***Annex 17***.

Results are presented in ***Annex 18*** and in the ***Figure 8*** and ***Table 6*** below. (b) (4)

(b) (4)

Figure 8 : (b) (4) *for Olive, dry extract, 20% (A) and Olive, dry extract, 10% (B) were the main peaks have been numbered according to their elution order and the numbers correspond to those of Table 6.*

Table 6 : Tentative identification of main peaks detected in samples of OLEANOX, 20% and 10% hydroxytyrosol (peak numbers correspond to those assigned in Figure 8)

(b) (4)

1.3.6 Analysis of potentially toxic processes (Trade secret)

(b) (4)

1.3.7 Disintegration and dissolution profile

Not applicable.

1.3.8 Shelf-life and conditions of storage (Trade secret)

A study has examined the stability of three batches of each of the 10% and 20% hydroxytyrosol extracts, in the following conditions:

(b) (4)

(b) (4)

.

Results are presented in the next tables and in ***Annex 19***, ***Annex 20***, ***Annex 21***, ***Annex 22***, ***Annex 23***, ***Annex 24***, ***Annex 25*** and ***Annex 26***). Based on these results, the shelf-life is proposed at 2 years (*pending additional results (expected for September 2023) for 3 years shelf-life*), when the olive extract is stored in (b) (4)

(b) (4)

Table 7: Stability study: accelerated conditions.

(b) (4)

Table 8: Stability study: intermediate conditions.

(b) (4)

Table 9 : Stability study: long-term conditions (only results for 24 months are available).

(b) (4)

(b) (4)

2. Dietary Supplement Manufacture (Recommended)

This section is not applicable (N/A) because the subject of this NDI, the olive fruit dry extract standardized in hydroxytyrosol contains no other ingredients. Therefore, the safety of the NDI is the same as the safety of the dietary supplement.

2.1. Raw materials

Not applicable

2.2. Formulation ingredients other than the NDI

Not applicable

2.3. Manufacturing process

Not applicable

2.4. Product specifications

Not applicable

2.5. Methods of analysis

Method of analysis of hydroxytyrosol is described in the section “NDI manufacture”.

2.6. Analysis of potentially toxic processes

Not applicable

2.7. Disintegration and dissolution profile

Not applicable

2.8. Shelf-life and conditions of storage

Not applicable

3. History Of Use Or Other Evidence Of Safety (Required)

3.1 History of use

Olive (*Olea europea* L.) has a long history of use. Olive, as table olive or olive oil, is consumed for centuries, mainly in Europe, but also in the rest of the world. Indeed, olive is now common. According to the International Olive Council, table olive consumption has grown from approximately 1000 tons in 1996 to more than 2600 tons in 2018. In the past 3 decades, table olive consumption in Europe has grown by about 70%. In the US and Canada, it has been estimated in 2009 that over 218,000 tons of table olives were consumed, with 97.8% consumed in the US (191,000 tons) (***Annex 27***). Spain is the largest supplier of table olives in the USA, followed by Greece, Morocco, Argentina, and Italy. Table olive consumption is decreasing in the US.

However, olives are mainly consumed as olive oil. USA and Canada consumption of olive oil increased from 2.8 million hectolitres in 2004 to 3.3 million hectolitres in 2009 (***Annex 27***). US consumption represented 88.7% of the olive oil consumption in North America (2.9 million hectolitres). Since 2009, olive oil consumption has continued to increase in the USA, with more than 400,000 tons consumed in 2021.

In its opinion on the safety of hydroxytyrosol as novel foods, EFSA reported concentrations of 3.5 mg/kg and 7.7 mg/kg of free hydroxytyrosol in virgin olive oil and extra virgin olive oil respectively, and about 550-650 mg/kg in olives depending on cultivars. Based on these values, it has been estimated that the mean daily intake of free hydroxytyrosol in European adults was 0.0105-0.28 mg/day through olive oil consumption and 51.33-12.95 mg/day through the consumption of table olive, for a 70-kg person.

In the US, the per capita consumption of processed olive (mainly olive oil) was 0.85 pounds in 2018/2019 (*i.e.* 385 g). If we consider an average hydroxytyrosol concentration of 5.6 mg/kg in olive oil, the daily hydroxytyrosol consumption is approximately 21.5 mg/day in the US.

In the US, *Olea europaea* L. is listed as ODI (old dietary ingredient), i.e. ingredients sold on the US market in dietary supplements before 15 October 1994. Moreover, a New Dietary Ingredient notification (NDI 351) has been submitted in 2006 for an olive fruit extract (100 mg/day, equivalent to 35 mg/day polyphenols, particularly 5 mg/day hydroxytyrosol and 0.3 mg/day tyrosol). FDA had objection on this notification due to the lack of manufacturing process and of a specific method of analysis of polyphenols.

A GRAS application (GRN726) has been submitted for an olive fruit extracts, standardized at ≥ 40% hydroxytyrosol. The extract can be used in 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, at a level of 5 to 10 mg of hydroxytyrosol per serving.

Regarding hydroxytyrosol, two GRAS notices have been submitted (GRN 600 and 876), one related to hydroxytyrosol produced by chemical synthesis (GRN 600) and the second one produced by fermentation of a culture of *E. coli* (GRN 876). These two applications were related to the use of hydroxytyrosol as additive. GRN 876 intended to use hydroxytyrosol as an antioxidant in bakery products, beverages, dairy products and substitutes, desserts, fats and oils, fruits 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 to 10 mg hydroxytyrosol per serving. Regarding the GRN 600

notice, hydroxytyrosol was intended for use as an antioxidant and antimicrobial in beverages, fats and oils, fresh and processed fruits and vegetables and juices, and gravy and sauces at use levels of 5.0 milligrams per serving.

There is therefore a large consumption of hydroxytyrosol in the US. Based on the GRAS notice GRN600, the estimated maximal consumption of hydroxytyrosol as additive in the proposed foodstuffs was 42.5 mg/person/day. This exposure does not consider the olive dietary intakes (as table olive or olive oil). By considering olive consumption, total hydroxytyrosol consumption may be greater than 65 mg/person/day.

3.1.1 Description of the relationship between the historically consumed material and the NDI or dietary supplement containing the NDI

As noted above, the historical use of olive demonstrates the fact that human beings have been exposed to all compounds present in the NDI, and notably hydroxytyrosol for centuries. Moreover, humans are widely exposed to hydroxytyrosol on a daily basis without adverse effects.

The NDI is obtained by using olive juice, *i.e.* the water fraction obtained during the production of olive oil. Hydroxytyrosol is enriched by using size-exclusion chromatography. No compound is added, and the NDI contains only substances naturally present in olive. Therefore, the safety of the NDI can be extrapolated from data on olive/olive oil and on hydroxytyrosol.

3.1.2 Describe identity information verifying the relationship between the historically consumed material and the NDI or dietary supplement containing the NDI

The specifications of the ingredient have been presented in ***Figure 6*** and ***Figure 7***, and ***Annex 3*** and ***Annex 4***. The product's identity and quality are standardized by parameters such as (b) (4) [redacted]

3.1.3 Historical conditions of use and cumulative exposure estimate for the historically consumed material

Table olive is historically consumed *ad libitum*, either as raw material, or after cooking. Olive can be also consumed as olive oil. There is no clear exposure estimate to olive and olive oil in the USA due to the lack of data.

However, in Europe, the EFSA FoodEx2 database provides an estimate of consumption to olive oil and table olives. These exposures are summarized in the next tables.

Table 10 : Mean and 95th percentile consumption (with range) of olive oil according to the FoodEx2 database (in g/day).

Population	Mean	95th percentile
Infant	1.75 g/day (0.05-6.86)	5.78 g/day (0.00-18.00)
Toddlers	2.47 g/day (0.05-13.62)	7.04 g/day (0.20-28.70)
Other children	3.67 g/day (0.02-26.66)	9.83 g/day (0.00-45.96)
Adolescents	6.05 g/day (0.04-31.95)	14.99 g/day (0.00-56.10)
Adults	6.13 g/day (0.03-33.78)	15.22 g/day (0.00-67.90)
Elderly	6.27 g/day (0.01-35.04)	16.02 g/day (0.00-66.70)
Very elderly	5.01 g/day (0.01-29.48)	14.10 g/day (0.00-48.50)

Table 11 : Mean and 95th percentile consumption (with range) of table olive (and similar) according to the FoodEx2 database (in g/day).

Population	Mean	95th percentile
Infant	0.01 g/day 0.02 (0.00-0.02)	0 g/day (0.0-0.0)
Toddlers	0.34 g/day (0.0-0.91)	2.04 g/day (0.0-8.16)
Other children	0.50 g/day (0.12-1.01)	2.88 g/day (0.00-9.76)
Adolescents	1.00 g/day (0.16-3.01)	5.07 g/day (0.0-11.86)
Adults	0.73 g/day (0.01-3.15)	3.96 g/day (0.0-16.35)
Elderly	0.25 g/day (0.10-0.43)	0 g/day (0.0-0.0)
Very elderly	0.20 g/day (0.10-0.31)	0 g/day (0.0-0.0)

Based on these values, and a mean content of free hydroxytyrosol in extra-virgin olive oil (highest value in extra-virgin olive oil, 7.7 mg/kg) and table olive (659.3 mg/kg) (*EFSA, 2017 [4728]*), the average and 95th percentile intake of hydroxytyrosol from these sources can be calculated as follow.

Table 12 : Calculated exposure to hydroxytyrosol through table olive and olive oil, in mg/day.

Population	Olive oil		Table olive		Total	
	Mean	95th percentile	Mean	95th percentile	Mean	95th percentile
Infant	0.013	0.078	0.007	0	0.020	0.078
Toddlers	0.019	0.134	0.224	0.457	0.243	0.591
Other children	0.028	0.278	0.330	0.949	0.358	1.227
Adolescents	0.047	0.698	0.659	3.343	0.706	4.041
Adults	0.047	0.718	0.481	1.906	0.528	2.624
Elderly	0.048	0.773	0.165	0	0.213	0.773
Very elderly	0.039	0.544	0.132	0	0.170	0.544

Based on these calculations, it can be estimated that:

- The daily consumption of hydroxytyrosol through the consumption of olive oil and table olive is below 5 mg/day in Europe. Daily consumption is lower in the USA due to the high consumption of olive in Europe, probably lower than 2 mg/day through olive-based products consumption.
- The use of hydroxytyrosol in food product in the US (GRAS notices GRN726, GRN600 and GRN876) is limited to 5 to 10 mg/serving.
- The proposed exposure to hydroxytyrosol from the current NDI in food supplements is 20 mg/day.
- The total exposure to hydroxytyrosol through all sources can be therefore estimated to be around 50-60 mg/day maximum.

3.1.4 Adverse events associated with historically consumed material

Only three cases of allergic reaction of olive products have been identified in the scientific literature (see part 3.2.2). Otherwise, olive and olive oil are totally safe for human consumption. No adverse events associated with olive or olive oil have been reported.

Olive and olive oil are recognized as beneficial for human health, olive oil being part of the Mediterranean diet. As reviewed by Gorzynik-Debicka *et al.* in 2018, olive oil is notably reported to have beneficial cardiovascular effects, through at least in part strong antioxidant effects.

3.1.5 Alternative rationale for reasonable expectation of safety based on history of use

Not applicable

3.2 Other evidence of safety

The intake of OLEANOX would be considered to be “intermittent” based on the information in this NDI notification.

Under FDA 2016 Section VI.B (“History of Use or Other Evidence of Safety”), question 19 recommends the following studies to assess the safety of an ingredient (like the olive fruit dry extract) where the dietary supplement containing the NDI is intended for intermittent use, the NDI has a documented history of safe daily chronic use, and the proposed use of the NDI leads to intake levels that are greater than the levels consumed historically:

(1) A two-study genotox battery

(2) A 14-day range-finding oral study to establish an MTD in an appropriate animal model;

(3) A 90-day subchronic oral study (same species as the range-finding study) to establish an MTD and a NOAEL for use in calculating the margin of safety;

(4) A single-dose or repeat-dose tolerability study in humans and/or an ADME study in animals, humans, or both; and

(5) A teratology study (rodent or non-rodent) (see note at end of list). Note: The teratology study is not needed if the product is labeled as not for use by women of childbearing age, pregnant or lactating women, or children 13 and younger.

As users of the dietary supplement should be healthy adults and the dietary supplement should not be used by children, during pregnancy or lactation, a teratology study would not be applicable.

However, due to the large set of data and history of consumption of olive/olive oil and hydroxytyrosol, the following strategy has been used for the safety assessment of the NDI:

- Two genotoxicity studies have been conducted to reinforce the data available in the literature,
- The sub-chronic oral toxicity is based on the literature since several studies have already provided strong data. No study has been conducted on the NDI.
- Human studies on olive fruit extract or hydroxytyrosol are used to substantiate the safety of the NDI.

Therefore, the current safety evaluation of OLEANOX aims to cover the gaps identified regarding genotoxicity and uses the large number of published data on olive extract or hydroxytyrosol to evaluate the other aspects of the toxicological evaluation. This work has been the object of a publication published in the peer-reviewed journal Toxicology Reports (Liamin *et al.*, 2023).

At last, a review of the composition of olive by-products has been performed to ensure the total absence of any nutritionally disadvantageous or dangerous compounds. The results of this review showed that olive mill wastewater and other relevant olive by-products contains various compounds found in olives. The three by-products described in this review present some similarities and also specificities of the different olive by-products. The main compounds found in olive mill wastewater are carbohydrates, proteins, minerals, and phenolic compounds. Olive pomace has strong similarities with

olive mill wastewater regarding phenolic compounds. Indeed, the major phenolic compounds found in olive pomace and olive mill wastewater are hydroxytyrosol, tyrosol and oleuropein. OS components are more different with compounds not identified in olive mill wastewater or olive pomace such as nüzhenide.

However, it is important to consider that the concentration of these different compounds varies between studies. This can be explained by the different oil extraction processes, the regions where the olives are grown, the cultivation methods and the analytical methods.

It is also important to consider that no compound associated with safety concern has been identified..

3.2.1 Safety study type

Pharmacokinetic

No study has examined the pharmacokinetic parameters of the olive fruit extract. However, several studies have already reported the pharmacokinetic parameters of hydroxytyrosol. Please see in part 2.10.4 the process of literature filtration.

In vitro studies

D'Antuono *et al.* (2016) have examined the bioaccessibility and intestinal absorption of biophenols from olive by using *in vitro* model. Bioaccessibility was variable depending on the compound studied: only 7% for luteolin, but 86.3% and 99% for hydroxytyrosol and tyrosol respectively. The mean bioaccessibility of polyphenols from table olive was 84.0 ± 5.9%. Hydroxytyrosol and tyrosol were rapidly absorbed by intestinal cells, with rapid excretion to the basolateral compartment. These results are in accordance with previous study that demonstrated hydroxytyrosol and tyrosol, but not conjugated form such as oleuropein, were rapidly absorbed in both jejunum and ileum according to results from transport and metabolism experiments in perfused rat intestinal model, and *in vitro* transepithelial migration assay (Corona *et al*., 2006).

Due to the importance of gut microbiota on the metabolism of food substances (Rowland *et al*., 2018), the effects of gut microbiota on the absorption of hydroxytyrosol have also been evaluated. Oleuropein, which can be hydrolyzed at gastric level, and is not absorbed in small intestine, is rapidly degraded by the colonic microflora resulting in the formation of hydroxytyrosol (Corona *et al*., 2006). Conjugated forms, such as oleuropein, can participate, via their hydrolysis in gastric juice or biotransformation by gut microbiota, to the increase of hydroxytyrosol levels absorbed.

Animal studies

In 1998, Bai *et al.* determined hydroxytyrosol plasma levels in male rats after oral administration of 55 mg/kg BW of hydroxytyrosol. Hydroxytyrosol was measurable 2 min after administration, with maximum levels between 5 and 10 min. The concentration then decreased rapidly within 60 min, and then slowly for 1-2h. Hydroxytyrosol was completely eliminated from the blood after 180 min.

Another study has examined the bioavailability of hydroxytyrosol and tyrosol in rats after intravenous injection or oral supplementation of radiolabeled compounds (Tuck *et al.,* 2001). Within 24 h after dosing, approximately 95% of radioactivity after intravenous injection or oral

supplementation of hydroxytyrosol in water was excreted in urine. 70% of the dose was excreted in urine within 24 h when hydroxytyrosol was orally administered in water. The majority was eliminated with 2 hours after iv injection and 4 hours after oral intake. The estimated bioavailability of hydroxytyrosol when given orally in oil and water was 99% and 75% respectively.

D'Angelo *et al*. (2001) investigated the metabolism of hydroxytyrosol in rats following a single intravenous administration of ^{14}C-labelled hydroxytyrosol (1.5 m/kg BW). Less than 8% of the injected radioactivity was present in the blood 5 min after injection, approximately 4% after 10 min, and 0.1% after 300 min. Thus, an accurate half-life in blood could not be measured, but was estimated to be within minutes. Regarding tissues, radioactivity was about 10 times higher in kidney than in other tissues. 90% of the radioactivity was excreted in urine collected up to 5 h. In all investigated tissues and in plasma, four metabolites were present already 5 min after injection (homovanillic alcohol, homovannilic acid, 2,3-dihydroxyphenylacetic acid, and 3,4-dihydroxyphenylacetaldehyde).

In their toxicity study, Christian *et al.* (2004) also investigated the pharmacokinetic parameters of hydroxytyrosol after administration of a hydrolyzed aqueous olive pulp extract (2.4% hydroxytyrosol). Results are summarized in the table hereafter. Results confirmed the rapid absorption of hydroxytyrosol.

Table 13: Hydroxytyrosol plasma pharmacokinetic parameters estimated after 90 days of treatment with an olive pulp extract in rats (from Christian et al., 2004).

Dosage (mg/kg/day)	No. of rats sampled*	Gender	C_{max} (ng/mL)	t_{max} (h)	t_{last} (h)	AUC_{last} (ng·h/mL)
1000	3	M	9.2	0.5	1	5.88
	3	F	8.29[a]	0.5[a]	4[a]	18.7[a]
1500	3	M	6.11	0.5	4	10.3
	3	F	7.53	2	2	9.84
2000	3	M	8.07	0.5	8	39.3
	3	F	10.2	0.5	8	42.7

*Samples from 3 rats/sex/time prior to last treatment and at 0.5, 1, 2, 4 and 8 hours post-treatment.
[a]Samples from only two rats; one questionable 8-hour outlier concentration excluded.

Still in rats, another study has examined the bioavailability of hydroxytyrosol and its derivatives (hydroxytyrosol acetate and 2,3-dihydroxyphenylacetic acid) at doses of 1 and 5 mg/kg by oral gavage (Dominguez-Perles *et al.,* 2017). Maximal plasma concentrations of hydroxytyrosol were measured at 30 min and 1 hours in male rats receiving 1 and 5 mg/kg respectively, and at 30 minutes for both doses in females. However, plasma max concentrations in hydroxytyrosol were dramatically higher in female than in male (16 ng/mL vs. less than 2 ng/mL in the 5 mg/kg group). This difference was explained by a faster transformation of hydroxytyrosol into hydroxytyrosol acetate in male rats than in female.

Peroulis *et al.* (2018) have also examined the bioavailability of polyphenols from olive. They showed that several phenolic compounds were detected in animal serum as soon as 1h after supplementation. This early appearance suggests an early gastric absorption for the authors. In a long-term study (16 weeks), they also shown that increased level of rutin, caffeic and p-coumaric acid were detected in animal serum. They level increased dose-dependently when the extract was added in the diet.

Human studies

Most of the available human studies have examined the bioavailability of polyphenols from olive oil (Miro-Casas *et al.,* 2001a; Miro-Casas *et al.,* 2001b; Miro-Casas *et al.,* 2003a; Miro-Casas *et al.*, 2003b; Visioli *et al.,* 2000; Vissers *et al.,* 2002). These studies have been extensively reviewed (see paragraph on reviews).

A study has examined the absorption of pure hydroxytyrosol (99.5%) obtained from olive mill wastewater in 10 subjects who received a single dose of 2.5 mg/kg BW (Gonzalez-Santiago *et al*., 2010). After administration, the plasma concentrations of free hydroxytyrosol and homovanillic alcohol increased in all subjects. Large variations among subjects have been noted on kinetic data. The Cmax were obtained after 13 min for hydroxytyrosol and 16.7 min for homovanillic alcohol. Estimated half-life was 8 min for both substances. The bioavailability of free hydroxytyrosol was 6.2% (2.4 to 11.8%). The main metabolites found in the 24h urine were homovanillic acid and 2,3-dihydroxyphenylacetic acid, both found in free form or as sulfate or glucuronide derivatives.

Another study has examined the bioavailability of hydroxytyrosol through the consumption of 100 mg olive (76.73 mg hydroxytyrosol and 19.48 mg tyrosol) in 7 healthy men (Kountouri *et al.,* 2007). Plasma concentrations in hydroxytyrosol, tyrosol, homovanillic acid, homovanillic alcohol and 2,3-dihydroxyphenylacetic acid increased after administration, with a maximal concentration reached after 1 h. Plasma polyphenols occurred mainly as glucuronide derivatives. Hydroxytyrosol was mainly excreted in urines in the form of homovanillic acid, 2,3-dihydroxyphenylacetic acid and homovanillic alcohol. Excretion rates of hydroxytyrosol, tyrosol and its metabolites were maximal at 0-4h after consumption of olives.

In another study, 21 volunteers have been recruited for a randomized, crossover, placebo-controlled and double-blinded study (Khymenets *et al.,* 2016). They were randomly assigned to receive a placebo, 5 or 25 mg/day hydroxytyrosol (by using an olive extract called hytolive). 24-hour urine samples were collected for one week after supplementation. A low inter-individual variability has been measured. Quantitatively, the total amount of hydroxytyrosol and its metabolites recovered in the urine accounted for 21% (25 mg dose) to 28% (5 mg dose) of the administered dose.

More recently, Siefer *et al.* (2018) have examined the absorption of hydroxytyrosol in humans, either from an olive extract or by using synthetic hydroxytyrosol (30 mg/day hydroxytyrosol in both groups). They used the excretion of hydroxytyrosol and homovanillic acid in urine to estimate the absorption of hydroxytyrosol. Results showed a similar increase in urinary homovanillic acid and hydroxytyrosol with pure hydroxytyrosol and olive extract, which was significant in comparison to placebo ($p < 0.0001$).

It should be noted that the pharmacokinetic parameters of hydroxytyrosol may be dependent on vehicle of administration or food matrix. Visioli *et al.* (2003) have shown that hydroxytyrosol excretion was higher after an administration in olive oil (44.2%) than in refined olive oil (23%) or in a yoghurt (5.8%).

Finally, a recent study has examined the pharmacokinetics and bioavailability of hydroxytyrosol in humans by using different food matrices (Aleman-Jimenez *et al.*, 2021). The study has been conducted in 20 healthy volunteers who ingested 5 mg hydroxytyrosol through different food matrices (extra virgin olive oil, and five fortified food matrices: refined olive oil, flax oil, grapeseed oil, margarine, and pineapple juice). After each intervention, each volunteer underwent a wash-out period of 96h to avoid interferences among the different food matrices.

A strong effect of food matrices on hydroxytyrosol pharmacokinetics has been noted. The intake of extra virgin olive exhibited significantly higher plasma concentrations after 30 min of oral intake (3.79 ng/mL) relative to the control. Regarding the hydroxytyrosol bioavailability, the intake of extra

virgin olive oil, as well as fortified refined olive, fax, and grapeseed oils provided significantly higher urinary contents (0.86, 0.63, 0.55, and 0.33 µg/mg creatinine, respectively) compared with basal urine, whereas hydroxytyrosol metabolites showed no significant changes. No differences were found between men and women

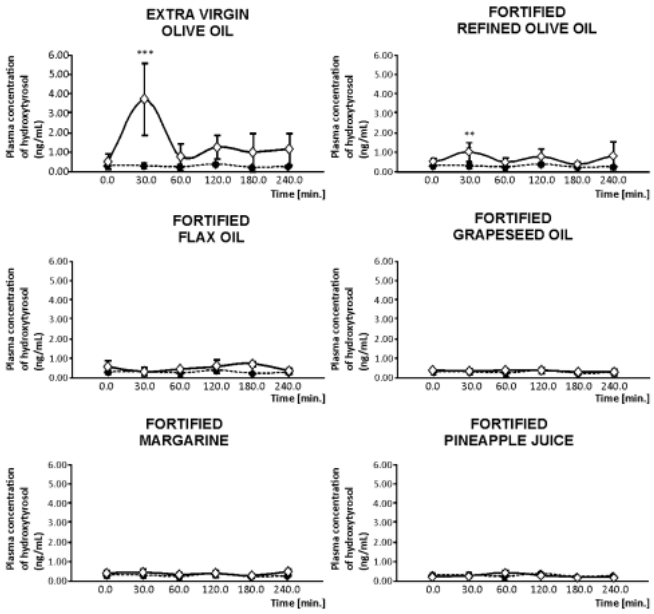


Figure 9 : Pharmacokinetics of hydroxytyrosol depending of the food matrices (from Aleman-Jimenez et al., 2021).

Therefore, the available data showed that the oily nature of the source is important to ensure a good bioavailability of hydroxytyrosol, with olive oil being the best matrix in comparison to other oils.

Reviews

In their review, Soni *et al.* (2006) indicated that oral bioavailability of hydroxytyrosol in olive oil and in an aqueous solution was reported as 99% and 75% respectively. Comparative studies reported greater urinary excretion in humans than in rats.

In 2008, another review has assessed the available data on the bioavailability of olive oil phenolic compounds in humans (De la Torre, 2008). The author concluded that hydroxytyrosol is well absorbed in the gastrointestinal tract and undergoes a first pass metabolism both in the gut and liver, which leads to the formation of sulfate and glucuronide conjugates. It was also noted that hydroxytyrosol is a dopamine metabolite, and body fluids contain hydroxytyrosol from exogenous and endogenous sources.

Other review have been published, providing similar positions (Fito *et al.,* 2007*;* Rodriguez-Morato *et al.,* 2016; Vissers *et al.,* 2004). More recently, Robles-Almazan *et al.* (2018) reviewed the toxicity and bioavailability of hydroxytyrosol. They indicate that the time required for the complete elimination from the body, both for hydroxytyrosol and its metabolites is approximately 6h in humans.

In the GRAS notice GRN 726 (Applicant: DSM) on a polyphenol preparation from olive fruits (40% hydroxytyrosol), the metabolic pathway of hydroxytyrosol has been proposed as follows:

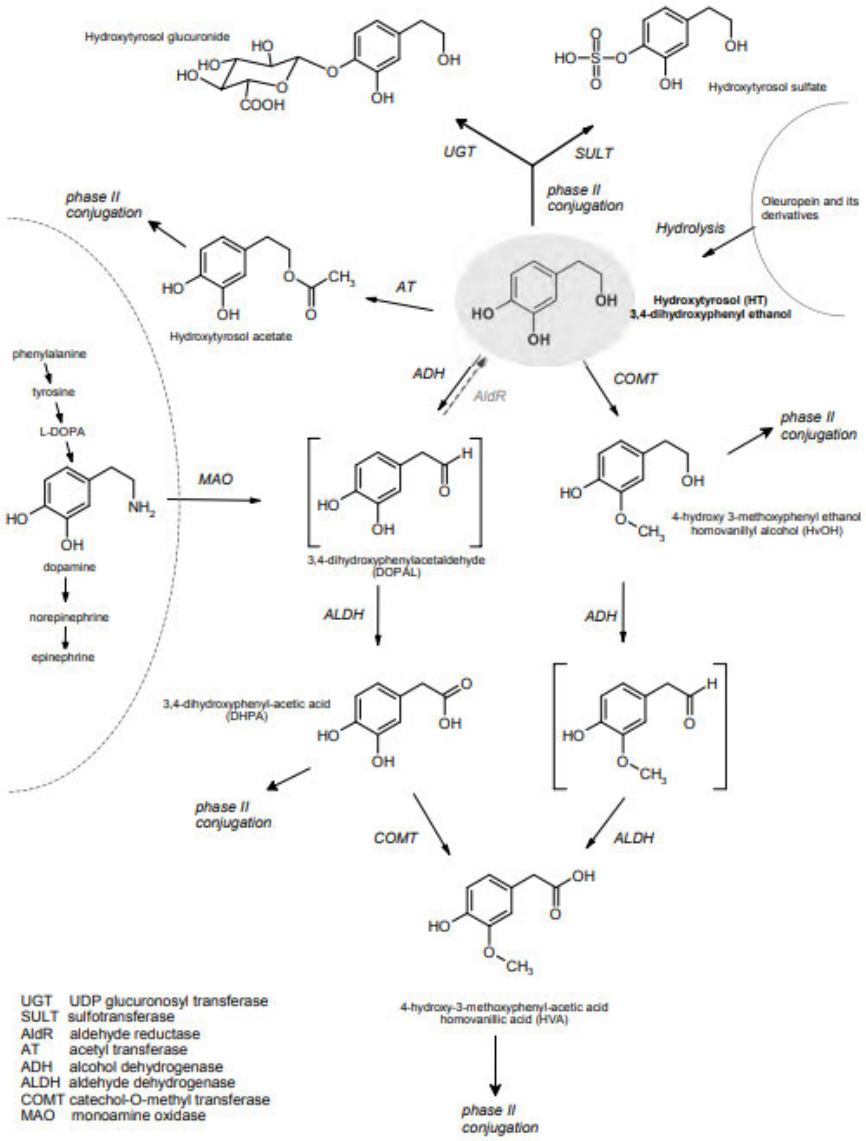


Figure 10: Metabolic pathways of hydroxytyrosol, as proposed in the GRAS notice GRN 726.

Conclusion

Overall, the available data on ADME parameters in rats and in humans indicates that hydroxytyrosol is quickly absorbed, with a C-max reached within few minutes, has a half-life of few minutes, and is eliminated by the kidneys as either free hydroxytyrosol, or in oxidized/conjugated forms (glucuronide and sulfate derivatives). The food matrix seems to have an effect on hydroxytyrosol bioavailability, with oily matrixes having a better absorption.

Genotoxicity

Bacterial reverse mutation test on OLEANOX (Trade secret)

A bacterial reverse mutation test has been conducted on the olive aqueous extract, standardized to 20% hydroxytyrosol (***Annex 28***). The study was compliant with the OECD 471 guidelines and followed Good Laboratory Practice.

(b) (4)

Therefore, the olive aqueous extract (20% hydroxytyrosol) had no mutagenic activity on the growth of the bacterial strains under the test conditions used in this study.

In vitro mammalian cell micronucleus test on OLEANOX (Trade secret)

One *in vitro* mammalian cell micronucleus test has been conducted on the olive aqueous extract (20% hydroxytyrosol) (***Annex 29***). The study was GLP compliant and followed OECD 487 guidelines.

(b) (4)

(b) (4) [redacted]

Therefore, the olive aqueous extract (20% hydroxytyrosol) did not cause statistically or biologically significant reproducible increases in the frequency of micronucleated mouse lymphoma L5178Y $TK^{+/-}$ 3.7.2 C cells in the performed experiments with and without metabolic activation. Therefore, the olive aqueous extract was considered as not being genotoxic in this tests system under the conditions of the study.

Additional published data on genotoxicity

Olive oil can be used as vehicle in mutagenicity studies. For instance, OECD 474 guideline on the mammalian erythrocyte micronucleus test or OECD 475 guideline on mammalian bone marrow chromosomal aberration test propose the use of olive oil as vehicle, highlighting the lack of genotoxicity of olive oil. Nevertheless, even if olive oil has no mutagenicity effect (Simula *et al.,* 1991), it may induce a cytotoxic effect, notably in mouse bone marrow cells, making difficult the assessment of the genotoxicity of the test compound.

A study has examined the genotoxicity of various olive extracts (Kirkland *et al.,* 2015). The authors analyzed olive extracts standardized to 15%, 36% or 40% hydroxytyrosol, and pure hydroxytyrosol. The genotoxicity of these extract has been assessed by using AMES tests, micronucleus test *in vitro* (CHO cells) and micronucleus test *in vivo* in rats. Results showed no significant genotoxicity effect in the AMES test. However, an increase in micronuclei has been measured in the micronucleus test *in vitro*, with both the pure hydroxytyrosol and the 15% extract. However, further experiments showed a high level of cytotoxicity and a capacity to induce the production of hydrogen peroxide, probably due to the interaction between hydroxytyrosol and medium compound, which may have induced micronuclei formation. In animals, the 15% extract did not induced any micronuclei up to 561 mg/kg. Regarding the 40% extract, a 90-day study showed an increase in bone marrow micronucleus formation frequencies at 250 and 500 mg hydroxytyrosol/kg BW/day. However, due to some protocol experiment discordances, these results were questionable, and acute micronucleus studies at doses up to 2000 mg hydroxytyrosol/kg BW reported negative results. Taking together, for the authors, these results demonstrate that olive extracts are not genotoxic at high doses *in vivo*, and any genotoxic risks for human consumers are negligible.

Several genotoxicity tests conducted with a hydrolyzed aqueous olive pulp extract have been

published in 2004 (Christian *et al.,* 2004). The authors have used an AMES test, an *in vitro* chromosome aberration assay test and an *in vivo* micronucleus assay in rats. Equivocal results have been obtained in the AMES test, with increases in the number of revertant for *Salmonella typhimurium* TA98 and TA100, mainly due to the increase of concomitant cytotoxicity and the presence of precipitates. The *in vitro* chromosome aberration test, conducted in CHO cells, showed an increase in the percentage of aberrant cells at 1000 µg/mL in the presence of S9 mix. The authors concluded that the extract was positive for the induction of chromosome aberration. Nevertheless, these results were not confirmed in the micronucleus assay conducted in rats where nonsignificant effect of the extract (up to 5000 mg/kg BW) on the number of micronuclei was observed. Moreover, this study showed a very high level of cytotoxicity, that can interfere with the results observed.

Additional studies have clearly shown the lack of genotoxicity of hydroxytyrosol. It has no genotoxic effect in the Drosophilia Wing-Spot test, and even an antigenotoxic effect (Anter *et al.,* 2010).

A study has examined the clastogenicity of hydroxytyrosol in a bone marrow chromosome aberration study in rat (Dolan *et al.,* 2014). The study has been conducted in accordance with the OECD 475 guideline. Hydroxytyrosol (up to 2000 mg/kg BW) did not induce chromosome aberrations in bone marrow cells and is therefore not clastogenic *in vivo*. However, no information about the test item was disclosed in the publication. However, EFSA considered this study as pertinent for the safety evaluation of the synthetic hydroxytyrosol, suggesting that pure hydroxytyrosol has been used.

Finally, the genotoxicity of hydroxytyrosol has been investigated the AMES test and the *in vitro* chromosomal aberration assay in human lymphocytes (Auñon-Calles *et al.,* 2013a). In absence of S9 mix, the highest tested dose (503.5 µg/mL) induced an increase in aberrant cells in the *in vitro* chromosomal aberration test. In presence of S9 mix, increases in aberrant cells were not for the medium and highest doses (287.7 and 503.5 µg/mL). Results of the AMES test showed no significant increase in the number of revertant colonies, with or without metabolic activation, for doses up to 5 µL/plate.

Overall, olive extracts or pure hydroxytyrosol have been associated with some genotoxic observations. Nevertheless, these effects have been noted only in vitro studies and main of these genotoxic effects have been linked to the concomitant cytotoxic potential of the product at highest doses and thus cannot be considered as true genotoxic effects. Moreover, these effects were observed only at doses highest than classical dose-range achieved by food consumption (Kirkland et al., 2015, Aunon-Calles et al., 2013). According to this,

Oral toxicity

In 2004, a publication has reported acute and sub-chronic toxicity studies conducted on a hydrolyzed aqueous olive pulp extract (Christian *et al.,* 2004). The tested product was HIDROX™, standardized to 2.4% hydroxytyrosol and 6% total phenolics. The product was solubilized in deionized water to a concentration of 200 mg/mL.

The acute toxicity study has been conducted in mice, which received 500, 1000 or 2000 mg/kg of the test product, in a single dose. No adverse effect has been noted, suggesting a LD_{50} greater than 2000 mg/kg (equivalent to 48 mg hydroxytyrosol/kg). Similar results have been obtained in rats in the same study. Moreover, the micronucleus assay described previously showed the lack of mortality or

clinical sign for a dose up to 5 g, suggesting that the LD_{50} is greater than 5 g (equivalent to 120 mg hydroxytyrosol).

In a 90-day sub-chronic toxicity study, 20 rats/group/sex received the extract at levels of 0, 1000, 1500 or 2000 mg/kg BW/day for 90 days. Except a slight decrease in body weight in male rats in the 1000 mg/kg BW group at days 71-78, which was no considered to be related to the test item, no change in body weight and organ weight has been measured. Hematological analysis were normal, except a dose-related increasing trend in the number of red blood cells in female, which was significant for the highest dose. However, the lack of any other change in the hematological analysis conducted the authors to consider the effect as a slight erythropoietic stimulation of the bone marrow without any toxicological consequences. It should be noted that markers of liver function (ALT, AST and sorbitol dehydrogenase) were reduced. Finally, histopathological analysis showed only some focal hyperplasia of the mucosal squamous epithelium of the limiting ridge of the forestomach with the highest dose, probably due to a local irritation by the large volume of viscous, granular formulation. The authors concluded to a NOAEL of 2000 mg/kg BW/day in this study.

All the safety studies on HIDROX^{TM} have been reviewed by Soni *et al*. in 2006. No additional data have been published. The authors concluded to the safety of the extract at levels up to 20 mg/kg BW/day (*i.e*. 1400 mg/day for 70-kg person, or 33.6 mg hydroxytyrosol).

The safety of a 35% hydroxytyrosol olive extract has been examined by Heilman *et al*. in 2015. The product has been administered to rat at doses of 0, 345, 691, and 1381 mg/kg for 90 days, corresponding to 0, 125, 250 and 500 mg/kg/day of hydroxytyrosol. Reductions in terminal body weight (9%), and a statistically significant reduction in body weight gain (17%, $p < 0.05$) at week 13 were observed in high dose males, as well as a statistically significant increase in relative weights of the liver, heart, and kidneys of high dose males and females. These changes were not accompanied by pathological or clinical observations and a trend towards reversal was observed in the recovery phase. H35 was well-tolerated and no toxicologically significant treatment-related changes were observed in condition and appearance of rats, neurobehavioral outcomes, motor activity assessments, functional observational battery, food intake, ophthalmoscopic examinations, hematology, clinical chemistry, urinalysis, necropsy findings, sperm parameters or estrus cycle. Due to the change in body weight gain, the NOAEL was 250 mg hydroxytyrosol/kg BW/day in this study.

A study (OECD compliant) has examined the acute (single dose), sub-acute (14 days) and sub-chronic (90 days) toxicity of an hydroxytyrosol-rich virgin oil extract (Rodriguez-Lara *et al.,* 2019). Even if the extract was prepared from oil and not from fruit, its hydroxytyrosol concentration (15%) is similar to the current Novel Food, and this study may therefore provide interesting information. The acute study (single dose of 300 mg/kg) did not highlight any side effect of the supplementation. Similarly, the sub-acute toxicity study (2000 mg/kg for 14 days) showed the lack of adverse effect of the tested product. In the sub-chronic test (100, 300 or 1000 mg/kg for 90 days, in the drinking water) showed no difference in body weight gain, water consumption, clinical signs, functional observations of sensory and moto reactivity, hematological and biochemical analyses, and macroscopic and microscopic histopathology.

A safety evaluation of olive phenolic compounds has been performed in rats (Farag *et al.,* 2003). The authors extracted total polyphenols and free phenolic acids from olive fruits or leaves. Extracts were administered for 7 weeks at 400, 800 or 1600 ppm. No effect of the total polyphenols or

free phenolic acids were noted at 400 and 800 ppm but increases in ALT and AST were noted at 1600 ppm. The highest doses also increased serum total lipids, as liver weight. No effect has been noted on kidney weight. Histopathological findings revealed severe damages to the tissues of the rat kidney and liver at 1600 ppm. Therefore, total polyphenols and free phenolic acids from olive fruits are safe in rats at doses up to 800 ppm/day for 7 weeks, but induced liver and kidney toxicity at 1600 ppm/day.

Other studies have examined the oral toxicity of hydroxytyrosol. In an acute toxicity test in rats, phosphatidyl-hydroxytyrosol at a dose of 2000 mg/kg BW had no adverse events (Martinez *et al.,* 2018). Moreover, in a 28-day study, phosphatidyl-hydroxytyrosol (2000 mg/kg BW) induced no mortality, adverse event, or any change in body weight, food consumption, clinical observation, blood biochemical, hematology, organ weight and histopathological findings.

Auñon-Calles *et al.* (2013b) have also examined the toxicity of pure hydroxytyrosol. The study was conducted in accordance with international guidelines, and notably OECD 408 guideline. Rats received for 13-weeks pure hydroxytyrosol by oral gavage at dose levels of 5, 50 and 500 mg/kg BW/day. No mortality has been recorded. Minimal decrease in locomotor activity has been recorded in male from the 500 mg/kg group, but also to a minor extent in the two other groups. The authors considered that these effects were not related to the test item and had no toxicological relevance. Moreover, body weight gain was slightly lower in male from the 500 mg/kg group. No difference in food consumption has been noted. Hematology analysis reported only minor changes among groups. Biochemistry findings reported lower glucose and creatinine and higher albumin and calcium values in the highest dose group. Higher AST values have been measured in male from all treated groups (significant only for the 5 and 50 mg/kg groups), but other liver markers were unaffected by the supplementation. No histopathological findings have been reported. The authors concluded to a NOAEL of 500 mg/kg in both female and male rats.

Reprotoxicity

In 2004, a publication has reported reprotoxicity studies conducted on an hydrolyzed aqueous olive pulp extract (Christian *et al.,* 2004). The tested product was HIDROX^{TM}, standardized to 2.4% hydroxytyrosol and 6% total phenolics. The product was solubilized in deionized water to a concentration of 200 mg/mL.

An oral dosage-range reproduction study has been conducted in rats, which received 0, 500, 1000, 1500 or 2000 mg/kg of the extract for 14 days before cohabitation and until necropsy. Males were euthanized after receiving 49 dosages of the extracts, and females after completion of a 22-day post-partum period. F1 generation pups were weaned 21 days post-partum. Two pups/sex/litter (a total of 80 rats per sex) were selected for a week of daily gavage dosage and recording of clinical signs, body weights and viability before being euthanized and necropsied on post-partum day 28. All other pups were subjected to gross necropsy on post-partum day 21. Only a small reduction in pup body weight (< 10%) on lactation days 7, 14 and 21 were noted in the 1000, 1500 and 2000 mg/kg groups. No other significant effect of the supplementation has been noted in this study.

In a follow-up investigation of this first study, the developmental toxicity of the extract has been investigated. Time-mated female rats were randomly divided in four groups. On days 6 to 20 of presumed gestation, the extract was administered at doses of 0, 1000, 1500 and 2000 mg/kg. On gestation day 21, one dam in the 2000 mg/kg/day group began to prematurely deliver its litter before scheduled Caesarean-sectioning and was euthanized. No abnormal findings were noted for this dam or its litter. All other rats survived until scheduled Caesarean-sectioning. No adverse clinical or

necropsy observations or significant differences in maternal body weights, body weight gains, gravid uterine weights, corrected maternal body weights or body weight gains or absolute or relative feed consumption values were noted between the groups. The extract treatment did not affect litter parameters at any of the doses. No treatment-related increases in gross external, soft tissue and skeletal fetal alterations (malformations or variations) were noted. A significantly increased mean number of corpora lutea of the 2000 mg/kg dose was well within the historical range of 14.5–20.1 per litter and was attributed to two females that had 27 or 30 corpora lutea. Based on these two studies, the authors concluded that the maternal and developmental NOAEL of the tested product was 2000 mg/kg BW/day, the highest dose administered.

Recently, Garcia-Contreras *et al.* (2019) have examined the effects of maternal hydroxytyrosol supplementation on placental gene expression and other parameters related to intrauterine growth restriction. The study has been conducted in sows. On day 35 of pregnancy, animals were fed with a diet fulfilling 50% of their daily requirements in order to affect fetal development including lower birth-weight in the newborns. Half of animals received also 1.5 mg of hydroxytyrosol per kg of feed from day 35 to day 100. Hydroxytyrosol had no consequences on maternal features, nor on fetal lengths and widths of body and head. However, fetuses in the hydroxytyrosol group showed lower mean body weights ($p < 0.005$) and lower mean weights of total viscerae, lungs, liver, pancreas and intestine ($p < 0.05$) than fetuses in the control group. Lower glucose and fructosamine plasma levels have been also noted in the hydroxytyrosol group. Therefore, even if this study has been conducted in a model of intrauterine growth restriction, results showed a possible effect of hydroxytyrosol on fetuses development.

Several human studies have examined the effects of olive extracts or hydroxytyrosol. Even if these studies were focused on the efficacy of such supplementations and not on their safety, they may provide useful information on the tolerability of olive extracts or hydroxytyrosol. These studies are summarized in the table hereafter.

Table 14 : Summary of human studies assessing the safety of olive fruit extract or hydroxytyrosol.

Authors	Design	Population	Supplementation	Safety results
Bitler *et al.*, 2007	Randomized, double-blinded and placebo-controlled study	n = 105 Subjects with osteoarthritis and rheumatoid arthritis.	**Group 1**: 400 mg/day of freeze-dried polyphenolic-rich olive extract (approximately 10 mg polyphenol/person/day) **Group 2**: placebo **Length of supplementation**: 8 weeks	No change in markers of hepatic function (AST, ASL, alkaline phosphatase, total bilirubin) or renal function (serum blood urea nitrogen, creatinine) have been noted. No side effect reported, except one case of heartburn in each group, alleviated by consuming the supplement with food.
Crespo *et al.*, 2015 **Tomé-Carneiro *et al.*, 2016**	Randomized, double-blinded, placebo-controlled and crossover trial	n = 21 Healthy subjects	**Group 1**: 5 mg/day hydroxytyrosol **Group 2**: 25 mg/day hydroxytyrosol **Group 3**: placebo **Length of supplementation**: 7 days each period, with 7-day washout between two supplementation period **Extract**: Hytolive®, an olive mill waste water extract	Both doses were well-tolerated, and no adverse effect was reported. No change in safety parameters: systolic and diastolic blood pressure, heart rate, plasma lipids, CRP, hepatic and renal markers.
Fukumitsu *et al.*, 2016	Randomized, double-blinded and placebo-controlled study	n = 20 Middle-age and elderly subjects with mild knee joint pain.	**Group 1**: olive fruit extract, providing 50 mg/day of maslinic acid **Group 2**: placebo **Length of supplementation**: 12 weeks **Extract**: olive fruit extract (500 mg/day), containing 10.7% maslinic acid, 57% gamma-cyclodextrin, 22.8% protein, 1.5% fat, 2.5% ash, 3.4% moisture and other minor components. No information about hydroxytyrosol.	No adverse effect. Only minor changes in blood parameters (increase in albumin, and reductions in alkaline phosphatase and chloride), but this were only minor changes, and values remained within normal range. These changes were not considered as adverse effects and were due only to biological variability.
Léger *et al.*, 2005	Uncontrolled study	n = 5 Men with type 1 diabetes	**Group 1**: 25 mg hydroxytyrosol the first day, and 12.5 mg the following 3 days **Length of supplementation**: 4 days **Extract**: olive mill waste water extract, 53% hydroxytyrosol and 13% tyrosol, doses calculated to provided 12.5 or 25 mg hydroxytyrosol	No side effect reported, and no change in safety parameters (blood glucose, HbA1c, plasma lipids, albumin, bilirubin, uric acid)

Lopez-Huertas *et al.*, 2017	Uncontrolled study	n = 14 Subjects with mild hyperlipidemia	**Group 1**: 45 mg hydroxytyrosol **Length of supplementation**: 8 weeks **Extract**: 99.5% hydroxytyrosol	The supplementation did not induce any change in weight, BMI and systolic/diastolic blood pressure. Biochemical parameters were unaffected, except a slight decrease un lactate dehydrogenase and a slight increase in creatine phosphokinase. Regarding hematology parameters, only a slight increase in mean corpuscular volume has been noted, whereas all other parameters remained constant. Urinalysis was normal. Vitamin and mineral assessment showed an increase in vitamin C concentration, whereas ferritin, serum and red blood cell folate were reduced.
Pais *et al.*, 2016	Randomized, double-blinded and placebo-controlled study	n = 36 Subjects at risk for arterial stiffness	**Group 1**: 250 mg of olive fruit extract, providing 50 mg hydroxytyrosol **Group 2**: 500 mg of the extract, providing 100 mg hydroxytyrosol **Group 3**: placebo **Length of supplementation**: 11 days **Extract**: Proliva®, a standardized olive fruit extract (20% hydroxytyrosol)	No difference in adverse events between supplementations and placebo. No change in markers of liver and kidney functions, and other biochemical analysis parameters (glucose, uric acid, cholesterol, triglycerides, HDL, LDL, urea, creatinine, blood urea nitrogen, total bilirubin, AST, ALT, lactate dehydrogenase, alkaline phosphatase, gamma-GT, total protein, albumin, globulin, albumin/globulin ratio, iron, calcium, phosphorus, sodium, potassium, chlorine). No effect of the olive extract supplementation on the cardio-ankle vascular index has been measured. Finally, CRP levels have not been affected.
Pecchioli *et al*., 2020	Randomized, double-blinded and placebo-controlled study	n = 80 Subjects with mild to moderate hypercholesterolemia	**Group 1**: nutraceutical product, containing polyunsaturated fatty acid, hydroxytyrosol, coenzyme Q10, folic acid, vitamin B12, vitamin E, piperine and red yeast rice **Group 2**: placebo **Length of supplementation**: 16 weeks	No adverse events or musculoskeletal disorders were reported in either group. No change in creatinine phosphokinase, AST, ALT or creatinine have been measured.
Peroulis *et al.*, 2019	Uncontrolled study	n = 35 Healthy subjects	**Phase 1**: meat product, without extract **Phase 2**: meat product, with the extract **Length of supplementation**: 4 weeks for each phase, with a 2-week washout between the two phases **Extract**: 3.75 mg polyphenols/day, equivalent to 20 g olive oil.	No effect of the supplementation on blood glucose and lipids levels. In a sub-group of patients with at least 2 biochemical or anthropometric elements of cardio-metabolic risk, the supplementation reduced glucose ($p < 0.002$) and insulin levels ($p = 0.03$). It also reduced total cholesterol ($p < 0.009$), triglycerides ($p < 0.005$), LDL-cholesterol ($p < 0.01$) and ox-LDL ($p< 0.01$). No modification of hematic markers (AST, ALT), and urea or creatinine levels. No side effect reported.

Siefer *et al*., 2018	Randomized, double-blinded, placebo-controlled and crossover trial	n = 30 Healthy subjects	**Group 1**: 30 mg/day hydroxytyrosol **Group 2**: olive fruit extract, providing 30 mg/day hydroxytyrosol **Group 3**: placebo **Length of supplementation**: 4 weeks each period, with 14-day washout between two supplementation period **Extract**: synthetic hydroxytyrosol, standardized to 20% hydroxytyrosol by using maltodextrin, or olive fruit extract, standardized to at least 15% hydroxytyrosol by using maltodextrin.	No change in total and HDL-cholesterol has been noted, but a significant reduction in LDL-cholesterol has been noted with synthetic hydroxytyrosol. No change in triglyceride concentration nor in blood pressure. None of the adverse event reported by participants (no difference among groups) was related to the test products.
Zullo *et al*., 2020	Observational pilot study	n = 60 Women (>18 years) with at least 4 episodes of vulvovaginal candidosis in the last 12 months	Capsules containing hydroxytyrosol, with also tea tree oil, tabebuia, Juglans regia, and copper. Two capsules/day for the first month, and then 1 capsule/day for 2 months. **Length of supplementation**: 3 months No information about hydroxytyrosol intake	Results suggested the efficacy of the product to prevent vulvovaginal candidosis. No adverse event and complications have been reported.

Authorities opinions

In 2011, EFSA has published a scientific opinion on the substantiation of health claims related to polyphenols in olive (EFSA, 2011 [2033]). Several health relationships have been examined by EFSA. Most of them have been rejected due to insufficient scientific substantiation or general and non-specific health effects. However, EFSA recognized the cause-and-effect relationship between the consumption of olive oil polyphenols (standardized by the content of hydroxytyrosol and its derivatives) and the protection of LDL particles from oxidative damage. To bear the claim, 5 mg of hydroxytyrosol and its derivatives in olive oil should be consumed daily. This claim has been limited by the European Commission to olive oil which contains at least 5 mg of hydroxytyrosol and its derivatives (e.g. oleuropein complex and tyrosol) per 20 g of oil.

More recently, EFSA has published an opinion on the safety of synthetic hydroxytyrosol (> 99% purity) as a novel food (EFSA, 2017 [4728]). The target population was the general population, excluding children under 3 years as pregnant and breastfeeding women. The NOAEL of hydroxytyrosol was 50 mg/kg BW/day in a 90-day sub-chronic toxicity study. It should be noted that higher dosage (500 mg/kg BW/day) induced changes in body weight (reduction) and organ weights (increase in relative weights). The applicant proposed the use of hydroxytyrosol in fish and vegetable oils (up to 2150 mg/kg) and in margarines (up to 175 mg/kg). This anticipated daily intake of hydroxytyrosol is in the range or even less than the exposure of hydroxytyrosol from the consumption of olive oil and olives. The safety margin between the anticipated intake and the NOAEL was 100 for children and 200 for adolescents and adults. Therefore, EFSA concluded that hydroxytyrosol was safe under the proposed uses and use levels.

In the US, *Olea europaea* L. is listed as ODI (old dietary ingredient), i.e. ingredients sold on the US market in dietary supplements before 15 October 1994. Moreover, a New Dietary Ingredient notification (NDI 351) has been submitted in 2006 for an olive fruit extract (100 mg/day, equivalent to 35 mg/day polyphenols, particularly 5 mg/day hydroxytyrosol and 0.3 mg/day tyrosol). FDA had objection on this notification due to the lack of manufacturing process and of a specific method of analysis of polyphenols.

A GRAS application (GRN726) has been submitted for an olive fruit extracts, standardized at ≥ 40% hydroxytyrosol. The extract can be used in 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, at a level of 5 to 10 mg of hydroxytyrosol per serving.

Regarding hydroxytyrosol, two GRAS notices have been submitted (GRN 600 and 876), one related to hydroxytyrosol produced by chemical synthesis (GRN 600) and the second one produced by fermentation of a culture of *E. coli* (GRN 876). These two applications were related to the use of hydroxytyrosol as additive. GRN 876 intended to use hydroxytyrosol as an antioxidant in bakery products, beverages, dairy products and substitutes, desserts, fats and oils, fruits 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 to 10 mg hydroxytyrosol per serving. Regarding the GRN 600 notice, hydroxytyrosol was intended for use as an antioxidant and antimicrobial in beverages, fats and oils, fresh and processed fruits and vegetables and juices, and gravy and sauces at use levels of 5.0 milligrams per serving.

Allergenicity (Trade secret)

To date, there is no data on the potential allergenicity of the olive fruit extract standardized to 10-20% hydroxytyrosol. (b) (4) [redacted] (***Annex 30*** and ***Annex 31***).

Olive pollen is a major allergen, inducing seasonal respiratory allergies in the Mediterranean countries (Esteve *et al.*, 2012; Weber *et al.,* 2013). In olive fruit, only one allergen has been described (Ole e 13), being included in the thaumatin-like family (Esteve *et al.,* 2012). It should be noted that cutaneous sensitization to olive oil also exist (Van Joost *et al.,* 1981).

Some case of allergy of olive fruit have been reported. A case of a 19-year-old woman has been reported in 2004, with a four-year history of episodes of facial, neck, and hands angioedema and intense palms itch. She tolerated olive oil. Prick test were positive to olive. The allergy was an IgE-mediated allergy (Azofra*, 2004*).

Another repot of food allergy due to olive has been published in 2009. This case a 28-yo man with palatal itching and generalized urticarial following ingestion of olive. Results of prick tests and prick-to-prick tests for olive were positive. An oral provocation test with olive oil did not cause symptoms (Unsel *et al.,* 2009). Racil *et al.* (2015) have also published a similar case, in a 48-year-old woman. They indicate that this woman was only the four cases reported with a diagnosis of “pollen-food olive-olive allergy”.

Therefore, oral allergy to olive fruit is very rare. Since the extract contains 1% protein, and due to these extremely rare cases of allergic reaction to olive, it can be concluded to the lack of allergic risk associated to the consumption of the extract.

3.2.2 Discussion of toxicity and conclusion

The table hereafter summarizes the toxicity studies conducted on olive extract and hydroxytyrosol.

Table 15 : Summary of toxicity studies on olive extract or hydroxytyrosol.

Reference	Experiments	Test products	Main results
Genotoxicity			
Proprietary data (Annex 28)	Bacterial reverse mutation test	20% hydroxytyrosol extract	Negative results
Proprietary data (Annex 29)	In vitro micronucleus test	20% hydroxytyrosol extract	Negative results
Kirkland *et al.*, 2015	AMES test *In vitro* micronucleus test *In vivo* micronucleus test	Olive extract standardized to 15, 26 or 40% hydroxytyrosol, o pure hydroxytyrosol	AMES test: negative results *In vitro* test: increase in micronuclei with the 15% extract and pure hydroxytyrosol *In vivo* test: increase in micronuclei in a 90-day study (250 / 500 mg/kg of the 40% extract), but no effect in an acute study
Christian *et al.*, 2004	AMES test *In vitro* chromosome aberration assay *In vivo* micronucleus test	HIDROX™, an hydrolyzed aqueous olive pulp extract, standardized to 2.4% hydroxytyrosol	AMES test: equivocal results In vitro test: increase in percentage of aberrant cells at 1000 µg/mL with metabolic activation, concluded to be positive for the induction of chromosome aberration. *In vivo* test: negative results
Anter *et al.*, 2010	Drosophilia Wing-Spot test	Pure hydroxytyrosol	Negative results
Dolan *et al.*, 2014	Bone marrow chromosome aberration study in rat	Pure hydroxytyrosol	Negative results
Auñon-Calles *et al.*, 2013a	AMES test *In vitro* chromosome aberration assay	Pure hydroxytyrosol	AMES test: negative results In vitro test: increase in aberrant cells for the highest tested dose (503.5 µg/mL) in absence of S9 mix, but negative results in presence of S9 mix.
Acute and sub-acute oral toxicity			
Christian *et al.*, 2004	Acute toxicity in mice and in rats (500, 1000, or 2000 mg/kg)	HIDROX™, an hydrolyzed aqueous olive pulp extract, standardized to 2.4% hydroxytyrosol	No adverse effect LD_{50} greater than 2000 mg/kg
Martinez *et al.*, 2018	Acute toxicity in rats 28-day repeated dose study in rats	Phosphatidyl-hydroxytyrosol	No adverse effect or significant changes in both studies LD_{50} greater than 2000 mg/kg BW
Rodriguez-Lara *et al.*, 2019	Acute toxicity in rats (300 mg/kg) 14-day repeated dose study in rats (2000 mg/kg)	Aqueous extract of olive oil, standardized to 15% hydroxytyrosol	No adverse effect or significant changes in both studies
Subchronic oral toxicity			
Christian *et al.*, 2004	90-day study in rats (0, 1000, 15000 or 2000 mg/kg)	HIDROX™, an hydrolyzed aqueous olive pulp extract, standardized to 2.4% hydroxytyrosol	NOAEL = 2000 mg/kg
Heilman *et al.*, 2015	90-day study in rats (0, 345, 691, or 1381 mg/kg of extract, corresponding to 0, 125, 250 or 500 mg/kg hydroxytyrosol)	Olive extract, standardized to 35% hydroxytyrosol	Reduction in body weight gain in male receiving the highest dose, associated to higher relative weights of the liver, heart and kidneys. No side effect

			NOAEL = 250 mg/kg BW of hydroxytyrosol
Rodriguez-Lara *et al.*, 2019	90-day study in rats (100, 300 or 1000 mg/kg)	Aqueous extract of olive oil, standardized to 15% hydroxytyrosol	No side effect NOAEL = 1000 mg/kg
Farag *et al.*, 2003	7-week toxicity study in rats (400, 800 or 1600 ppm)	Total polyphenols or free phenolic acids extracted from olive fruits	Increase in ALT and AST at 1600 ppm, associated with increases in serum lipids, liver weight, and severe damages to the liver and kidney tissues. No side effects of the 400 and 800 ppm doses.
Auñon-Calles *et al.*, 2013b	90-day study in rats (5, 50, or 500 mg/kg)	Pure hydroxytyrosol	Increase in AST values in males (5 and 50 mg/kg groups), but no other change in liver parameters. NOAEL = 500 mg/kg
Reprotoxicity and developmental toxicity			
Christian *et al.*, 2004	Toxicity of reproduction and developmental toxicity in rats (0, 500, 1000, 1500 or 2000 mg/kg)	HIDROX™, an hydrolyzed aqueous olive pulp extract, standardized to 2.4% hydroxytyrosol	Only a slight reduction in pup body weight (< 10%) was noted on lactation days 7, 14 and 21 for doses > 1000 mg/kg. No other adverse effect. NOAEL = 2000 mg/kg
Garcia-Contreras *et al.*, 2019	Toxicity in a sow model of intrauterine growth restriction	Pure hydroxytyrosol	Possible effects on fetuses development, with lower body weight and lower mean weights of total viscerae, lungs, liver, pancreas and intestine.
Human studies			
Bitler *et al.*, 2007 **Pais *et al.*, 2016** **Peroulis *et al.*, 2019** **Fukumistu *et al.*, 2016** **Siefer *et al.*, 2018**	Various design (see table 15)	Olive extracts, standardized in polyphenols (up to 500 mg extract per day, corresponding to 100 mg/day hydroxytyrosol)	No side effects, notably no change in blood pressure, hepatic markers, and renal markers.
Crespo *et al.*, 2015 **Pecchioli *et al.*,2020** **Léger *et al.*, 2005** **Lopez-Huertas *et al.*, 2017** **Siefer *et al.*, 2018**	Various design (see table 15)	Pure hydroxytyrosol (up to 45 mg/day)	

In addition, it should be noted that several reviews on the toxicity of olive extracts and hydroxytyrosol have confirmed the lack of safety concern associated with oral consumption of hydroxytyrosol (Liamin *et al*., 2023; Robles-Almazan *et al.,* 2018; Soni *et al.,* 2006).

The NOAEL determined based on repeated oral dosing in rodents, were reported to be between 48 and 250 mg hydroxytyrosol/kg BW/day. As presented previously, some effects were noted in *in vivo* studies after oral supplementation with more than 50 mg/kg BW/day of hydroxytyrosol. In addition, human studies have demonstrated the safety of olive fruit extract, for doses up to 100 mg/day of hydroxytyrosol from fruit extract (Pais et al., 2016).

Considering the 50 mg/kg BW/day as point of departure in animals and applying uncertainties factors allowing to consider the interspecies variations and the interindividual variations within human, an acceptable daily intake of hydroxytyrosol for human is proposed to be 0.5 mg/kg BW/day. Considering that the product olive dry extract provides up to 20% hydroxytyrosol, a dose of 2.5 mg/kg BW/day of olive dry extract titrated to 20% hydroxytyrosol can be considered as safe.

Therefore, the proposed daily intake of the Novel Food (20 mg hydroxytyrosol/day) can be therefore considered as totally safe.

It should be reminded that the current NDI is proposed to be use in food supplements for adults only. Pregnant/breastfeeding women and children will not consume the NDI.

3.2.3 Alternative rationale for reasonable expectation of safety based on other evidence of safety

Not applicable.

4. Basis For Concluding That the New Dietary Ingredient Will Reasonably Be Expected To Be Safe For Use in the Dietary Supplement (Required)

The available data demonstrate a high degree of safety associated with the consumption of the olive fruit dry extract standardized in hydroxytyrosol, other olive fruit extract, olive fruit and olive oil, and the primary active constituent hydroxytyrosol. No significant adverse effect can be attributed to the olive fruit dry extract or to hydroxytyrosol.

- The scientific literature provides conflicting results regarding the genotoxicity of olive fruit extract and hydroxytyrosol. The available studies have used different preparation and different hydroxytyrosol levels, up to 40%. However, genotoxic effects observed *in vitro* are concomitant with cytotoxic and cytostatic effects and have been observed in high doses. Moreover, *in vitro* studies did not support this genotoxic effect and demonstrated the lack of genotoxicity of the NDI.
- Acute oral toxicity studies on olive extracts have demonstrated the safety of such extracts, with a LD_{50} greater than 2000 mg/kg for an olive fruit extract standardized to 2.4% hydroxytyrosol (LD_{50} expressed for hydroxytyrosol greater than 48 mg/kg).
- In sub-chronic toxicity, various NOAEL have been reported: 691 mg/kg for a 35% hydroxytyrosol extract (Heilman *et al.,* 2015), 1000 mg/kg for a 15% extract (Rodriguez-Lara *et al*., 2019) and 2000 mg/kg for a 2.4% extract (Christian *et al.,* 2004). This corresponds to 250 mg/kg, 150 mg/kg and 48 mg/kg when expressed in hydroxytyrosol, respectively.
- Regarding pure hydroxytyrosol, the available sub-chronic toxicity study reported a NOAEL of 500 mg/kg (Auñon-Calles *et al*., 2013b).
- 10 clinical trials have examined the effects of either olive fruit extract or pure hydroxytyrosol in humans, without reporting any significant safety concern. A study has notably no adverse effect, no change in markers of liver and kidney function, or in other biochemical analysis parameters after the consumption for 11 days of 500 mg/day of an olive fruit extract providing 100 mg/day hydroxytyrosol (Pais *et al.,* 2016).
- The historical consumption of table olive and olive oils also clearly substantiate the safe use of the NDI. Notably, olive allergy is rare and does not represent a safety concern.

Therefore, based on the available evidence, it could be concluded that the olive fruit dry extract standardized in hydroxytyrosol is devoid of any genotoxicity, oral toxicity, allergenicity and side effects. The NDI is therefore safe under the proposed conditions of use.

4.1 Determination of the No-Observed-Adverse-Effect-Level (NOAEL) or Lowest-Observed Adverse Effect Level (LOAEL)

Several studies have provided different NOAEL. The table hereafter summaries these studies.

Table 16 : Summary of the NOAEL identified in the different studies.

Authors	Test product	NOAEL (of the test product)	NOAEL (expressed for hydroxytyrosol)
Christian *et al.*, 2004	HIDROX™, an hydrolyzed aqueous olive pulp extract, standardized to 2.4% hydroxytyrosol	2000 mg/kg	48 mg/kg
Heilman *et al.*, 2015	Olive extract, standardized to 35% hydroxytyrosol	691 mg/kg	250 mg/kg
Auñon-Calles *et al.*, 2013b	Pure hydroxytyrosol	500 mg/day	500 mg/day
Rodriguez-Lara *et al.*, 2091	Aqueous extract of olive oil, standardized to 15% hydroxytyrosol	1000 mg/kg	150 mg/kg

4.2 Determination of safety factor

The safety evaluation of the NDI and of hydroxytyrosol is based on 1) several sub-chronic toxicity studies conducted in rodents, and 2) on human studies assessing the effects of supplementation with olive extract standardized in hydroxytyrosol or of pure hydroxytyrosol, 3) pharmacokinetic results showing a rapid absorption of hydroxytyrosol, a rapid metabolization, and the lack of any bio-accumulation of hydroxytyrosol or its derivatives.

Therefore, the safety factors in the case of the NDI should include:

- An uncertainty factor for interspecies variation (factor = 10)
- An uncertainty factor for inter-human variability (factor = 10)

Due to the availabilities of human data showing the safety of 50 mg/day of hydroxytyrosol in humans, it appears not necessary to include addition uncertainty factors. Therefore, the total safety factor is proposed to be 100.

4.3 Determination of the Acceptable Daily Intake (ADI)

Based on a safety factor of 100, the ADI can be calculated as described in the next table. The proposed consumption of the NDI corresponds to an intake of 0.2857 mg/kg BW/day of hydroxytyrosol.

Table 17 : Calculation of the ADI based on the different studies identified.

Authors	Test product	NOAEL hydroxytyrosol	ADI in humans
Christian *et al.*, 2004	HIDROX™, an hydrolyzed aqueous olive pulp extract, standardized to 2.4% hydroxytyrosol	48 mg/kg	0. 48 mg/kg BW/day
Heilman *et al.*, 2015	Olive extract, standardized to 35% hydroxytyrosol	250 mg/kg	2.50 mg/kg BW/day
Auñon-Calles *et al.*, 2013b	Pure hydroxytyrosol	500 mg/day	5 mg/kg BW/day
Rodriguez-Lara *et al.*, 2091	Aqueous extract of olive oil, standardized to 15% hydroxytyrosol	150 mg/kg	1.50 mg/kg BW/day

4.4 Determination of Estimated Daily Intake (EDI) and the EDI/ADI Ratio

The dosage recommendation for the olive fruit dry extract is 20 mg/day of hydroxytyrosol, which corresponds to either 200 mg/day of the 10% extract or 100 mg/day of the 10% extract. FDA assumes an average of 70 kg BW for an adult, and therefore the EDI for the NDI would be (expressed for hydroxytyrosol):

Hydroxytyrosol EDI = 20 mg/day ÷ 70 kg = 0.2857 mg/kg BW/day

It should be noted that this calculation corresponds to the highest consumption and does not take into account the intermittent consumption of the NDI. If this intermittent consumption was considered, the EDI would be lower.

The EDI/ADI ratio is between 0.057 and 0.59 depending on the study (presented in the ***Table 17***). It should be noted that all the studies provide an EDI/ADI ratio < 1.

4.5 Determination of margin of safety

In accordance with FDA recommendations, the margin of safety for a dietary ingredient is calculated by dividing the NOAEL in animal studies by the EDI of the dietary ingredient. A margin of safety of 100-fold means the doses shown to be without adverse effects in animals or humans are 100 times greater than the levels that would be consumed from the use of the dietary supplement.

As shown previously, different NOAEL have been identified by hydroxytyrosol. The table below calculates the margin of safety for each study.

Table 18 : Calculation of the margin of safety based on the different studies identified.

Authors	Test product	NOAEL hydroxytyrosol	Margin of safety
Christian *et al.*, 2004	HIDROX™, an hydrolyzed aqueous olive pulp extract, standardized to 2.4% hydroxytyrosol	48 mg/kg	168
Heilman *et al.*, 2015	Olive extract, standardized to 35% hydroxytyrosol	250 mg/kg	875
Auñon-Calles *et al.*, 2013b	Pure hydroxytyrosol	500 mg/day	1750
Rodriguez-Lara *et al.*, 2091	Aqueous extract of olive oil, standardized to 15% hydroxytyrosol	150 mg/kg	525

Therefore, depending on the studies, the margin of safety is in the range of 168 to 1750. It should be noted that these margins of safety do not take into account the intermittent exposure to the NDI. Margin of safety would be higher if this intermittent consumption was considered.

4.6 Safety narrative and conclusion

The margin of safety between the NOAEL and the EDI for hydroxytyrosol is between 168 and 1750 depending on the study considered. This margin of safety does not take into consideration the intermittent exposure of the NDI and is therefore underestimated. It would be higher if the intermittent exposure was considered. Moreover, the dietary supplements containing the olive fruit dry extract will be intended for intermittent use and olive fruit extract has a documented history of safe daily chronic use. These margins of safety are adequate however to conclude that hydroxytyrosol and olive fruit extract are reasonably expected to be safe under its intended conditions of use in dietary supplements.

The above estimates are very conservative because it assumes that a person consumes dietary supplement containing the NDI at the maximum recommended daily dose of 100 to 200 mg depending on the hydroxytyrosol concentration (all equivalent to 20 mg hydroxytyrosol per day) at once. In reality, the person may consume less than the maximum recommended daily dose, and manufacturers of finished dietary supplements may choose to deliver this amount in a single serving or divide this total into correspondingly reduced serving amounts taken in two or three divided doses. As previously discussed, the half-life of hydroxytyrosol is only few minutes, thus being rapidly absent from the body and metabolized. Therefore, given the conservatism of the estimation and the proposed uses of the olive fruit dry extract standardized in hydroxytyrosol, the margins of safety are adequate to conclude that hydroxytyrosol and the NDI are reasonably expected to be safe under its intended conditions of use in dietary supplements.

4.7 Alternative basis for reasonable expectation of safety

Not applicable.

5. Reference List

Anter J, Campos-Sánchez J, Hamss RE, et al. Modulation of genotoxicity by extra-virgin olive oil and some of its distinctive components assessed by use of the Drosophila wing-spot test. Mutat Res. 2010;703(2):137-142. https://www.sciencedirect.com/science/article/abs/pii/S1383571810002901

Auñon-Calles D, Canut L, Visioli F. Toxicological evaluation of pure hydroxytyrosol. Food Chem Toxicol. 2013b;55:498-504. https://www.sciencedirect.com/science/article/abs/pii/S0278691513000689

Auñon-Calles D, Giordano E, Bohnenberger S, Visioli F. Hydroxytyrosol is not genotoxic in vitro. Pharmacol Res. 2013a;74:87-93. https://www.sciencedirect.com/science/article/abs/pii/S1043661813000996

Azofra J. Olive allergy. Allergy. 2004;59(5):559. https://onlinelibrary.wiley.com/doi/abs/10.1046/j.1398-9995.2003.00124.x

Gorzynik-Debicka M, Przychodzen P, Cappello F, Kuban-Jankowska A, Marino Gammazza A, Knap N, Wozniak M, Gorska-Ponikowska M. Potential Health Benefits of Olive Oil and Plant Polyphenols. Int J Mol Sci. 2018 Feb 28;19(3):686. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5877547/

Rowland I, Gibson G, Heinken A, Scott K, Swann J, Thiele I, Tuohy K. Gut microbiota functions: metabolism of nutrients and other food components. Eur J Nutr. 2018 Feb;57(1):1-24. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5847071/

Domínguez-Perles R, Auñón D, Ferreres F, Gil-Izquierdo A. Physiological linkage of gender, bioavailable hydroxytyrosol derivatives, and their metabolites with systemic catecholamine metabolism. Food Funct. 2017 Dec 13;8(12):4570-4581. doi: 10.1039/c7fo01124e. PMID: 29119177. https://pubs.rsc.org/en/content/articlelanding/2017/FO/C7FO01124E

Alemán-Jiménez C, Domínguez-Perles R, Medina S, Prgomet I, López-González I, Simonelli-Muñoz A, Campillo-Cano M, Auñón D, Ferreres F, Gil-Izquierdo Á. Pharmacokinetics and bioavailability of hydroxytyrosol are dependent on the food matrix in humans. Eur J Nutr. 2021 Mar;60(2):905-915. https://link.springer.com/article/10.1007/s00394-020-02295-0

Fitó M, de la Torre R, Farré-Albaladejo M, Khymenetz O, Marrugat J, Covas MI. Bioavailability and antioxidant effects of olive oil phenolic compounds in humans: a review. Ann Ist Super Sanita. 2007;43(4):375-381. https://www.nature.com/articles/1601917

Bai C, Yan X, Takenaka M, Sekiya K, Nagat T. Determination of synthetic hydroxytyrosol in rat plasma by GC-MS. J Agric Food Chem 1998, 46:3998-4001. https://pubs.acs.org/doi/10.1021/jf980451r

Bitler CM, Matt K, Irving M, Hook G, Yusen J, Eagar F, Kirschner K, Walker B, Crea R. Olive extract supplement decreases pain and improves daily activities in adults with osteoarthritis and decreases plasma homocysteine in those with rheumatoid arthritis. Nutrition Research 2007;27:470-477. https://www.sciencedirect.com/science/article/abs/pii/S0271531707001315

Christian MS, Sharper VA, Hoberman AM, et al. The toxicity profile of hydrolyzed aqueous olive pulp

extract. Drug Chem Toxicol. 2004;27(4):309-330. https://www.tandfonline.com/doi/abs/10.1081/dct-200039714?journalCode=idct20

Contreras MDM, Gómez-Cruz I, Romero I, Castro E. Olive Pomace-Derived Biomasses Fractionation through a Two-Step Extraction Based on the Use of Ultrasounds: Chemical Characteristics. Foods. 2021 Jan 7;10(1):111. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7825784/

Corona G, Tzounis X, Assunta Dessì M, Deiana M, Debnam ES, Visioli F, Spencer JP. The fate of olive oil polyphenols in the gastrointestinal tract: implications of gastric and colonic microflora-dependent biotransformation. Free Radic Res. 2006 Jun;40(6):647-58. https://www.tandfonline.com/doi/abs/10.1080/10715760500373000?journalCode=ifra20

Crespo MC, Tomé-Carneiro J, Burgos-Ramos E, et al. One-week administration of hydroxytyrosol to humans does not activate Phase II enzymes. Pharmacol Res. 2015;95-96:132-137. https://www.sciencedirect.com/science/article/abs/pii/S1043661815000572

D'Angelo S, Manna C, Migliardi V, et al. Pharmacokinetics and metabolism of hydroxytyrosol, a natural antioxidant from olive oil. Drug Metab Dispos. 2001;29(11):1492-1498. https://dmd.aspetjournals.org/content/29/11/1492

D'Antuono I, Garbetta A, Ciasca B, et al. Biophenols from Table Olive cv Bella di Cerignola: Chemical Characterization, Bioaccessibility, and Intestinal Absorption. J Agric Food Chem. 2016;64(28):5671-5678. https://pubs.acs.org/doi/10.1021/acs.jafc.6b01642

de la Torre R. Bioavailability of olive oil phenolic compounds in humans. Inflammopharmacology. 2008;16(5):245-247. https://link.springer.com/article/10.1007%2Fs10787-008-8029-4

Dolan LC, Hofman-Hüther H, Amann N. Hydroxytyrosol: lack of clastogenicity in a bone marrow chromosome aberration study in rats. BMC Res Notes. 2014;7:923. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4301881/

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies),Turck D, Bresson J-L, Burlingame B, Dean T, Fairweather-Tait S, Heinonen M, Hirsch-Ernst KI,Mangelsdorf I, McArdle HJ, Naska A, Neuh€auser-Berthold M, Nowicka G, Pentieva K, Sanz Y, Siani A,Sj€odin A, Stern M, Tome D, Vinceti M, Willatts P, Engel K-H, Marchelli R, P€oting A, Poulsen M,Schlatter J, Turla E and van Loveren H, 2017. Scientific opinion on safety of hydroxytyrosol asa novel food pursuant to Regulation (EC) No 258/97. EFSA Journal 2017;15(3):4728. https://www.efsa.europa.eu/en/efsajournal/pub/4728

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA); Scientific Opinion on the substantiation of health claims related to polyphenols in olive and protection of LDL particles from oxidative damage (ID 1333, 1638, 1639, 1696, 2865), maintenance of normal blood HDL-cholesterol concentrations (ID 1639), maintenance of normal blood pressure (ID 3781), “anti-inflammatory properties” (ID 1882), “contributes to the upper respiratory tract health” (ID 3468), “can help to maintain a normal function of gastrointestinal tract” (3779), and “contributes to body defences against external agents” (ID 3467) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Journal 2011;9(4):2033. https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/j.efsa.2011.2033?fbclid=IwAR1QMnEz-ELp6AvHcu3UlxEYWjhB1H9bFGg_ScUzNJ6zE19tESoZ_LQ6EsI

Esteve C, Montealegre C, Marina ML, García MC. Analysis of olive allergens. Talanta. 2012;92:1-14. https://www.sciencedirect.com/science/article/abs/pii/S0039914012000227

Farag RS, El-Baroty GS, Basuny AM. Safety evaluation of olive phenolic compounds as natural antioxidants. Int J Food Sci Nutr. 2003;54(3):159-174. https://www.tandfonline.com/doi/abs/10.1080/0963748031000136306

Fukumitsu S, Villareal MO, Aida K, Hino A, Hori N, Isoda H, Naito Y. Maslinic acid in olive fruit alleviates mild knee joint pain and improves quality of life by promoting weight loss in the elderly. J Clin Biochem Nutr 2016;1-6. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5110940/

Garcia-Contreras C, Vazquez-Gomez M, Barbero A, et al. Polyphenols and IUGR Pregnancies: Effects of Maternal Hydroxytyrosol Supplementation on Placental Gene Expression and Fetal Antioxidant Status, DNA-Methylation and Phenotype. Int J Mol Sci. 2019;20(5):1187. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6429121/

Ghanbari R, Anwar F, Alkharfy KM, Gilani AH, Saari N. Valuable nutrients and functional bioactives in different parts of olive (Olea europaea L.)-a review. Int J Mol Sci. 2012;13(3):3291-3340. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3317714/

González-Santiago M, Fonollá J, Lopez-Huertas E. Human absorption of a supplement containing purified hydroxytyrosol, a natural antioxidant from olive oil, and evidence for its transient association with low-density lipoproteins. Pharmacol Res. 2010;61(4):364-370. https://www.sciencedirect.com/science/article/abs/pii/S1043661809003119

GRAS Notice GRN 726. Summary of information supporting the generally recognized as safe (GRAS) status of elaVidaTM (a polyphenol preparation from olive fruits) for use as an ingredient in selected foods. Submitted in 2017 by DSM Nutritional Products, LLC. https://www.fda.gov/media/111827/download

Heilman J, Anyangwe N, Tran N, Edwards J, Beilstein P, López J. Toxicological evaluation of an olive extract, H35: Subchronic toxicity in the rat. Food Chem Toxicol. 2015;84:18-28. https://www.sciencedirect.com/science/article/abs/pii/S0278691515300156

Khymenets O, Crespo MC, Dangles O, Rakotomanomana N, Andres-Lacueva C, Visioli F. Human hydroxytyrosol's absorption and excretion from a nutraceutical. J Funct Food. 2016;23:278-282. https://hal.inrae.fr/hal-02639403/document

Kirkland D, Edwards J, Woehrle T, Beilstein P. Investigations into the genotoxic potential of olive extracts. Mutat Res Genet Toxicol Environ Mutagen. 2015 Jan 1;777:17-28. https://www.sciencedirect.com/science/article/abs/pii/S1383571814002903

Kountouri AM, Mylona A, Kaliora AC, Andrikopoulos NK. Bioavailability of the phenolic compounds of the fruits (drupes) of Olea europaea (olives): impact on plasma antioxidant status in humans. Phytomedicine. 2007;14(10):659-667. https://www.sciencedirect.com/science/article/abs/pii/S0944711307001055

Léger CL, Carbonneau MA, Michel F, et al. A thromboxane effect of a hydroxytyrosol-rich olive oil

wastewater extract in patients with uncomplicated type I diabetes. Eur J Clin Nutr. 2005;59(5):727-730. https://www.nature.com/articles/1602133.pdf

Liamin M, Lara MP, Michelet O, Rouault M, Quintela JC, Le Bloch J. Olive juice dry extract containing hydroxytyrosol, as a nontoxic and safe substance: Results from pre-clinical studies and review of toxicological studies. Toxicol Rep. 2023 Feb 12;10:245-260.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9958074/

Lopez-Huertas E, Fonolla J. Hydroxytyrosol supplementation increases vitamin C levels in vivo. A human volunteer trial. Redox Biol. 2017;11:384-389. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5219601/

Martínez MA, Ares I, Martínez-Larrañaga MR, et al. Acute and repeated dose (28 days) oral safety studies of phosphatidyl-hydroxytyrosol. Food Chem Toxicol. 2018;120:462-471. https://www.sciencedirect.com/science/article/abs/pii/S0278691518304915

Miró Casas E, Farré Albadalejo M, Covas Planells MI, Fitó Colomer M, Lamuela Raventós RM, de la Torre Fornell R. Tyrosol bioavailability in humans after ingestion of virgin olive oil. Clin Chem. 2001a;47(2):341-343. https://academic.oup.com/clinchem/article/47/2/341/5639105

Miró-Casas E, Covas MI, Farre M, Fito M, Ortuno J, Weinbrenner T, Roset P, De la Torre R. Hydroxytyrosol disposition in humans. Clinical chemistry, 2003a, 49 :945-952. https://academic.oup.com/clinchem/article/49/6/945/5641783

Miró-Casas E, Covas MI, Fitó M, Farré-Albadalejo M, Marrugat J, de la Torre R. Tyrosol and hydroxytyrosol are absorbed from moderate and sustained doses of virgin olive oil in humans. Eur J Clin Nutr. 2003b;57(1):186-190. https://www.nature.com/articles/1601532.pdf

Miró-Casas E, Farré Albaladejo M, Covas MI, et al. Capillary gas chromatography-mass spectrometry quantitative determination of hydroxytyrosol and tyrosol in human urine after olive oil intake. Anal Biochem. 2001;294(1):63-72. https://www.sciencedirect.com/science/article/abs/pii/S0003269701951607

Pais P, Villar A, Rull S. Impact of a Proprietary Standardized Olive Fruit Extract (SOFE) on Cardio- Ankle Vascular Index, Visual Analog Scale and C-Reactive Protein Assessments in Subjects with Arterial Stiffness Risk. Drugs R D. 2016;16(4):355-368. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5114205/

Pecchioli V, Cicero AFG, Lomartire N, Gemmiti MP, Colangeli A, Germanò R, Corsi MP, Germanò GIW. A double-blind, placebo-controlled clinical trial to assess the effects of a combined nutraceutical on endothelial function in patients with mild-to-moderate hypercholesterolaemia. Arch Med Sci Atheroscler Dis. 2020 May 7;5:e36-e42. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7277437/

Peroulis N, Androutsopoulos VP, Notas G, et al. Significant metabolic improvement by a water extract of olives: animal and human evidence. Eur J Nutr. 2019;58(6):2545-2560. https://link.springer.com/article/10.1007%2Fs00394-018-1807-x

Racil H, Loukil M, Ferah Y, et al. The Pollen-Food Olive-Olive Syndrome. Tunis Med. 2015;93(5):326-327. http://www.latunisiemedicale.com/article-medicale-tunisie.php?article=2815

Robles-Almazan M, Pulido-Moran M, Moreno-Fernandez J, Ramirez-Tortosa C, Rodriguez-Garcia C,

Quiles JL, Ramirez-Tortosa M. Hydroxytyrosol: Bioavailability, toxicity, and clinical applications. Food Res Int. 2018 Mar;105:654-667.
https://www.sciencedirect.com/science/article/abs/pii/S0963996917308220

Rodríguez-Lara A, Mesa MD, Aragón-Vela J, et al. Acute/Subacute and Sub-Chronic Oral Toxicity of a Hidroxytyrosol-Rich Virgin Olive Oil Extract. Nutrients. 2019;11(9):2133.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6770357/

Rodríguez-Morató J, Boronat A, Kotronoulas A, Pujadas M, Pastor A, Olesti E, Pérez-Mañá C, Khymenets O, Fitó M, Farré M, de la Torre R. Metabolic disposition and biological significance of simple phenols of dietary origin: hydroxytyrosol and tyrosol. Drug Metab Rev. 2016 May;48(2):218-36.
https://www.tandfonline.com/doi/abs/10.1080/03602532.2016.1179754?journalCode=idmr20

Rowland I, Gibson G, Heinken A, Scott K, Swann J, Thiele I, Tuohy K. Gut microbiota functions: metabolism of nutrients and other food components. Eur J Nutr. 2018 Feb;57(1):1-24.

https://link.springer.com/article/10.1007/s00394-017-1445-8

Siefer S, Wacker R, Wilhelm M, Schoen C. Absorption of hydroxytyrosol from different sources and its impact on lipid status in human subjects. J Nutri Med Diet Care 2018;4(1):025.
https://clinmedjournals.org/articles/jnmdc/journal-of-nutritional-medicine-and-diet-care-jnmdc-4-025.pdf?jid=jnmdc

Simula AP, Priestly BG. Influence of vegetable oil vehicles on bone-marrow proliferation in the mouse micronucleus test. Mutat Res. 1991;261(1):83-84.
https://www.sciencedirect.com/science/article/abs/pii/016512189190101Q

Soni MG, Burdock GA, Christian MS, Bitler CM, Crea R. Safety assessment of aqueous olive pulp extract as an antioxidant or antimicrobial agent in foods. Food Chem Toxicol. 2006;44(7):903-915.
https://www.sciencedirect.com/science/article/abs/pii/S0278691506000159

Tomé-Carneiro J, Crespo MC, Iglesias-Gutierrez E, et al. Hydroxytyrosol supplementation modulates the expression of miRNAs in rodents and in humans. J Nutr Biochem. 2016;34:146-155.
https://www.sciencedirect.com/science/article/abs/pii/S0955286316301085

Tuck KL, Freeman MP, Hayball PJ, Stretch GL, Stupans I. The in vivo fate of hydroxytyrosol and tyrosol, antioxidant phenolic constituents of olive oil, after intravenous and oral dosing of labeled compounds to rats. J Nutr. 2001;131(7):1993-1996. https://academic.oup.com/jn/article/131/7/1993/4686878

Unsel M, Ardeniz O, Mete N, et al. Food allergy due to olive. J Investig Allergol Clin Immunol. 2009;19(6):497-499. http://www.jiaci.org/issues/vol19issue6/vol19issue06-12.htm

van Joost T, Smitt JH, van Ketel WG. Sensitization to olive oil (olea europeae). Contact Dermatitis. 1981;7(6):309-310. https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1600-0536.1981.tb04086.x?sid=nlm%3Apubmed

Visioli F, Galli C, Bornet F, et al. Olive oil phenolics are dose-dependently absorbed in humans. FEBS Lett. 2000;468(2-3):159-160. https://febs.onlinelibrary.wiley.com/doi/full/10.1016/S0014-5793%2800%2901216-3?sid=nlm%3Apubmed

Visioli F, Galli C, Grande S, et al. Hydroxytyrosol excretion differs between rats and humans and depends on the vehicle of administration. J Nutr. 2003;133(8):2612-2615. https://academic.oup.com/jn/article/133/8/2612/4687986

Vissers MN, Zock PL, Katan MB. Bioavailability and antioxidant effects of olive oil phenols in humans: a review. Eur J Clin Nutr. 2004;58(6):955-965. https://www.nature.com/articles/1601917

Vissers MN, Zock PL, Roodenburg AJ, Leenen R, Katan MB. Olive oil phenols are absorbed in humans. J Nutr. 2002;132(3):409-417. https://www.nature.com/articles/1601917.pdf

Weber RW. Allergen of the month-European olive. Ann Allergy Asthma Immunol. 2013;110(4):A19. https://www.annallergy.org/article/S1081-1206(13)00131-2/fulltext

Zullo MA, Schiavi MC, Di Pinto A, Prata G, Scudo M, Luffarelli P, Oliva C. Efficacy and safety of oral administration of a product based on hydroxytyrosol as preventive therapy for recurrent vulvo-vaginal candidosis: a prospective observational pilot study. Eur Rev Med Pharmacol Sci. 2020 Jul;24(13):7427-7432. https://www.europeanreview.org/article/21911

6. Comments

No comments.