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Safety of hydroxytyrosol as a novel food pursuant to Regulation (EC) No 258/97

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Abstract

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on hydroxytyrosol, which is chemically synthesised, as a novel food (NF) pursuant to Regulation (EC) No 258/97. The information provided on the composition, specifications, batch-to-batch variability, stability and production process of the NF is sufficient and does not raise concerns about the safety of the NF. The applicant intends to add hydroxytyrosol to fish and vegetable oils up to 215 mg/kg and to margarines up to 175 mg/kg. The target group is the general population which excludes children under 36 months of age, pregnant women and breastfeeding women. Considering the no observed adverse effect level (NOAEL) of 50 mg/kg body weight per day from a subchronic oral toxicity study with the NF and the maximum anticipated daily intake for the NF, the margin of exposure (MoE) would result in 100 for children (3–9 years of age) and at least 200 for adolescents, adults (excluding pregnant and breastfeeding women) and elderly. Taking into account that the anticipated daily intake of the NF would be in the range of or even less than the exposure of hydroxytyrosol from the consumption of olive oils and olives, which has not been associated with adverse effects, and considering the similar kinetics of hydroxytyrosol in rats and humans, the Panel considers that the MoE for the NF at the intended uses and use levels is sufficient for the target population. The Panel concludes that the novel food, hydroxytyrosol, is safe under the proposed uses and use levels.

Keywords: hydroxytyrosol, novel food, ingredient, safety

Requestor: European Commission following an application by Seprox Biotech

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Summary

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on hydroxytyrosol as a novel food (NF) submitted pursuant to Regulation (EC) No 258/97. The assessment, which follows the methodology set out in Commission Recommendation 97/618/EC, is based on the data supplied in the original application, the initial assessment by the competent authority of Spain, the concerns and objections of a scientific nature raised by the other Member States and the responses of the applicant.

The NF that is subject of this application is hydroxytyrosol, which is chemically synthesised. The information provided on the composition, specifications, batch-to-batch variability, stability and production process of the NF is sufficient and does not raise concerns about the safety of the NF.

The applicant intends to add hydroxytyrosol to fish and vegetable oils up to 215 mg/kg and to margarines up to 175 mg/kg. The target group is the general population which excludes children under 36 months of age, pregnant women and breastfeeding women.

The main dietary sources of hydroxytyrosol are olive oils and table olives. The daily intake of hydroxytyrosol from the consumption of olive oils and olives has been calculated based on a mean content of free hydroxytyrosol in these foods. The Panel notes that in olives and olive oils hydroxytyrosol is not only available in a free form, but also in the conjugated forms of oleuropein and oleuropein-aglycones, and that a wide range of content of free hydroxytyrosol and its conjugated forms in these foods, which depends on the variety and degree of ripeness of olives, has been reported. Since bioavailability studies on polyphenols from olives and olive oils show that also hydroxytyrosol from oleuropein and oleuropein-aglycone is bioavailable, the Panel considers that the total systemic exposure to hydroxytyrosol from olive oils and olives is higher than the exposure estimated on the basis of the content of free hydroxytyrosol only (approximately six and three times higher for olive oils and olives, respectively, based on a mean content of oleuropein and oleuropein-aglycone).

Overall, the provided data on the kinetics suggest that in both rats and humans hydroxytyrosol is quickly absorbed, has a half-life time of a few minutes and is eliminated by the kidneys as either free hydroxytyrosol or in oxidised or conjugated forms (glucuronide and sulfate derivatives).

Taking into account the intended use levels, the Panel considers that the consumption of the NF is not nutritionally disadvantageous.

Based on the studies provided, the Panel concludes that there is no concern with regard to potential genotoxicity of the NF. The NF was tested at dose levels of 5, 50, or 500 mg/kg body weight (bw) per day in a subchronic 90-day oral toxicity study in rats. Based on changes in body and organ weights in the highest dose group tested in this study, the Panel considers the dose of 50 mg/kg bw per day as the no observed adverse effect level (NOAEL). The findings in this 90-day study with the NF were supported by the observations in another 90-day oral toxicity study in rats with an olive extract which delivered different doses of hydroxytyrosol.

Considering the NOAEL of 50 mg/kg bw per day in the subchronic oral toxicity study with the NF and the maximum anticipated daily intake for the NF, the margin of exposure (MoE; i.e. the ratio between the NOAEL and the maximum anticipated daily intake of the NF) would result in 100 for children (3–9 years of age) and at least 200 for adolescents, adults (excluding pregnant and breastfeeding women) and elderly.

Taking into account that the anticipated daily intake of the NF would be in the range of or even less than the exposure of hydroxytyrosol from the consumption of olive oils and olives, which has not been associated with adverse effects, and considering the similar kinetics of hydroxytyrosol in rats and humans, the Panel considers that the MoE for the NF at the intended uses and use levels is sufficient for the target population.

The Panel concludes that the novel food, hydroxytyrosol, is safe under the proposed uses and use levels.

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1. Introduction

1.1. Background and Terms of Reference as provided by the European Commission

On 3 October 2014, the company Seprox Biotech submitted a request under Article 4 of the Novel Food Regulation (EC) No 258/97[1] to place on the market hydroxytyrosol as a novel food (NF).

On 9 March 2015, the competent authority of Spain forwarded to the Commission its initial assessment report, which came to the conclusion that hydroxytyrosol meets the criteria for acceptance of a novel food defined in Article 3(1) of Regulation (EC) No 258/97.

On 10 April 2015, the Commission forwarded the initial assessment report to the other Member States. Several of the Member States submitted comments or raised objections.

The concerns of a scientific nature raised by the Member States can be summarised as follows:

- Clarifications were requested on some results of the batch analyses of the NF.
- Clarifications were requested on the laboratories' accreditation to internationally recognised system for the analyses of the NF.
- More information was requested on the degradation products of the NF in vegetable oils at elevated temperature (e.g. cooking conditions).
- Information was requested on the combined intake of hydroxytyrosol from the background diet and food supplements.
- The reason for excluding breastfeeding women and children under 36 months of age was requested. Some Member States requested that also pregnant women should be excluded from the target population.
- The NF could be nutritionally disadvantageous when supplemented to oils which would replace olive oil in the consumers' diet.
- The *in vitro* human lymphocyte chromosomal aberration study reported that hydroxytyrosol (with or without metabolic activation) induced an increase in the number of cells with structural chromosome aberrations. In line with the EFSA guideline on testing for genotoxicity (EFSA, 2011), an *in vivo* micronucleus test with the NF is required.
- Some Member States commented that the findings in the 90-day subchronic toxicity study were not adequately addressed.
- Request of additional toxicological data on developmental safety and safety on long-term consumption of the NF is needed.
- One Member State commented on the lack of human studies with the NF.
- More information on protein residues should be provided.

In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002[2], the European Food Safety Authority is asked to carry out the additional assessment for hydroxytyrosol as a NF in the context of Regulation (EC) No 258/97.

The European Food Safety Authority (EFSA) is asked to carry out the additional assessment and to consider the elements of a scientific nature in the comments raised by the other Member States.

2. Data and methodologies

2.1. Data

The assessment of the safety of this NF is based on data supplied in the original application, the initial assessment by the competent authority of Spain, the concerns and objections of the other Member States and the responses of the applicant.

[1] Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. OJ L 43, 14.2.1997, p. 1–6.

[2] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24.

In accordance with Commission Recommendation 97/618/EC[3], hydroxytyrosol is allocated to Class 1.2, i.e. 'foods and food components that are single chemically defined substances or mixtures of these which are not obtained from plants, animals or microorganisms that have been genetically modified and whose source has no history of food use in the Community'. The data are required to comply with the information required for novel foods of Class 1.2, i.e. structured schemes I, II, III, IX, XI, XII and XIII of Commission Recommendation 97/618/EC. In the current scientific opinion, these structured schemes are listed in Sections 3.1–3.8. The intention is to add the NF to oils and fats. This assessment concerns only risk that might be associated with consumption of the NF under the proposed conditions of use, and is not an assessment of the efficacy of the NF with regard to any claimed benefit.

2.2. Methodologies

The assessment follows the methodology set out in Commission Recommendation 97/618/EC of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients and the preparation of initial assessment reports under Regulation (EC) No 258/97 of the European Parliament and of the Council.

3. Assessment

3.1. Specifications of the Novel Food (NF)

The NF which is the subject of this application is hydroxytyrosol.

The structural formula of hydroxytyrosol (IUPAC name: 4-(2-hydroxyethyl)-1,2-benzenediol); $C_8H_{10}O_3$; CAS no: 10597-60-1; molecular weight: 154.16 Da) is presented in Figure 1. Synonyms for this compound are 3-hydroxytyrosol, 3,4-hydroxyphenylethanol (DOPET), dihydroxyphenylethanol, 2-(3,4,dihydroxyphenyl)-ethanol and 3,4-dihydroxyphenolethanol. Hydroxytyrosol is naturally present in foods, in particular in olive oils and in table olives.

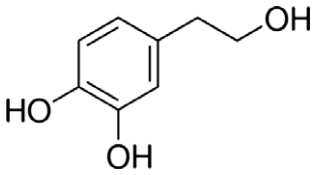

Figure 1: Structural formula of hydroxytyrosol

The NF is manufactured via chemical synthesis. The NF is described as a slightly hydrated (< 4%) yellow viscous liquid with a hydroxytyrosol purity of > 99% (high-performance liquid chromatography (HPLC)). The identity of hydroxytyrosol has been verified using liquid chromatography with tandem mass spectrometry (LC–MS/MS), proton nuclear magnetic resonance (^{1}H-NMR) and carbon-13 nuclear magnetic resonance (^{13}C-NMR). The specifications of the NF are presented in Table 1. The applicant has identified four possible residual organic by-products in six batches by using both HPLC with ultraviolet (UV) detection at 280 nm and liquid chromatography–mass spectrometry (LC–MS). The main impurity is hydroxytyrosol acetate (< 0.3% as analysed by HPLC-UV; 0.2–0.8% as analysed by LC–MS), whereas the other organic compounds consist of homovanillic alcohol, iso-homovanillic alcohol, 3-methoxy-4-hydroxyphenylglycol (overall, < 0.1% as analysed by HPLC-UV; < 0.4% as analysed by LC–MS). Additional impurities that can be present at very low concentrations are residual heavy metals (lead, cadmium and mercury) and residual solvents which include ethyl acetate, isopropanol, methanol and tetrahydrofuran.

[3] Commission Recommendation 97/618/EC: Commission Recommendation of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients and the preparation of initial assessment reports under Regulation (EC) No 258/97 of the European Parliament and of the Council. OJ L 253, 16.9.19971 p. 1–36.

Test results for six batches of NF show compliance with the proposed specifications (Table 2).

Table 1: Specifications of the NF

Parameter	Specifications	Assay method
Description	Slightly yellow viscous liquid	Visual
Odour	Characteristics	Organoleptic
Taste	Slightly bitter	Organoleptic
Solubility (water)	Miscible in water	Saturation
Moisture	< 4%	Thermogravimetry analysis (HMA) or Karl Fischer method
pH	3.5–4.5	1 M water solution
Chromatographic purity	> 99.0%	HPLC-UV
Hydroxytyrosol content	> 95.0%	Calculated[(a)]
Hydroxytyrosol acetate	< 0.3%	HPLC-UV
Others	< 0.1%	HPLC-UV
Acetic acid	< 0.4%	HPLC/refractive index detector
Inorganics/heavy metals		
Lead	< 0.03 ppm	ICP-MS
Cadmium	< 0.01 ppm	ICP-MS
Mercury	< 0.01 ppm	ICP-MS
Total inorganics[(b)]	< 0.3%	ICP-MS for cations; IC for anions
Residual organic solvents		
Ethyl acetate	< 25.00 ppm	Head Space/GC/MS
Isopropanol	< 2.50 ppm	Head Space/GC/MS
Methanol	< 2 ppm	Head Space/GC/MS
Tetrahydrofuran	< 0.01 ppm	Head Space/GC/MS

HMA: halogen moisture analyser; HPLC: high-performance liquid chromatography; GC: gas chromatography; IC: ion-exchange chromatography; ICP-MS: inductively coupled plasma mass spectrometry; MS: mass spectrometry.
(a): Calculated: 100 – (hydroxytyrosol acetate) – (moisture Karl Fischer) – (total inorganics) – (acetic acid) – (residual organic solvents).
(b): It includes boron, sodium, potassium, calcium, chromium, iron, nickel, chloride, nitrate, nitrite, sulfate.

Table 2: Analysis of six batches of the NF

Parameter	Batches					
	E012 1820 16	E012 0305 21	E012 162225 24	E012 1617 26	E012 121318 29	E012 0716 34
Chromatographic purity HPLC-UV	99.51	99.42	99.43	99.41	99.43	99.34
Hydroxytyrosol content calculated[(a)]	97.67	95.51	95.45	95.90	95.17	95.41
COA[(b)]	97.38	95.90	95.62	96.26	95.98	95.92
Hydroxytyrosol acetate HPLC-UV (%)	0.18	0.20	0.25	0.13	0.10	0.26
Others HPLC-UV (%)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Acetic acid (%)	0.32	0.30	0.25	0.11	0.41	0.28
Moisture Karl Fischer (%)	1.56	3.88	3.95	3.71	4.07	3.87
Thermogravimetry analysis (HMA) (%)	2.13	3.52	3.81	3.57	3.36	3.42

Parameter	Batches					
	E012 1820 16	E012 0305 21	E012 162225 24	E012 1617 26	E012 121318 29	E012 0716 34
Inorganics/heavy metals (ppm)						
Boron	n.a.	12.7	4.3	6.5	5.0	8.7
Sodium	413.0	376.8	378.4	491.5	514.0	425.1
Potassium	811.6	175.0	202.3	168.3	753.1	480.0
Calcium	77.5	65.3	139.4	58.5	173.4	202.6
Cadmium	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Chromium	0.02	0.16	0.07	0.13	0.04	<0.01
Iron	1.07	4.1	0.91	3.3	0.81	2.09
Mercury	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Nickel	0.08	0.08	0.02	0.03	0.02	0.02
Lead	0.03	0.03	0.02	0.02	< 0.01	< 0.01
Chloride	955.3	344.3	161.0	441.8	820.5	325.9
Nitrate	12.9	22.7	1.2	24.0	1.4	1.8
Nitrite	7.6	< 1.0	0.14	< 1.0	0.25	0.2
Sulfate	431.6	107.9	141.6	169.8	273.2	192.0
Total inorganics						
ppm	2,710.7	1,109.07	1,029.36	1,363.88	2,541.72	1,638.41
%	0.27	0.11	0.1	0.14	0.25	0.17
Acetic acid (%)	0.32	0.30	0.25	0.11	0.41	0.28
Residual organic solvents (ppm)						
Ethyl acetate	22.65	7.51	17.71	8.09	0.91	0.91
Acetone	< 0.01	1.85	< 0.01	< 0.01	< 0.01	< 0.01
Isopropanol	1.8	0.28	1.10	2.27	0.92	0.65
Methanol	< 0.01	< 0.01	< 0.01	1.93	< 0.01	< 0.01
Tetrahydrofuran	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Total organic solvents						
ppm	24.45	9.64	18.81	12.29	1.83	1.56
%	0.0024	0.0010	0.0019	0.0012	0.0002	0.0002

HPLC-UV: high-performance liquid chromatography-ultraviolet; HMA: halogen moisture analyser; n.a.: not applicable.
(a): Calculated: 100 – (hydroxytyrosol acetate) – (moisture Karl Fischer) – (total inorganics) – (acetic acid) – (residual organic solvents).
(b): COA: (hydroxytyrosol HPLC-UV) – (moisture HMA).

The applicant stated that the laboratories providing the analysis of hydroxytyrosol and the residual impurities are accredited, but no certificates have been provided. However, the analyses of hydroxytyrosol have been carried out using a validated HPLC-UV method.

The Panel considers that the information provided on the composition, the specification and the batch-to-batch variability of the NF is sufficient and does not raise concerns about the safety of the NF.

3.1.1. Stability of the NF

The stability of hydroxytyrosol under the proposed conditions of storage (4°C, dark, and protected atmosphere) has been demonstrated in five batches for up to 20–23 months. The control parameters analysed were: colour, moisture and purity (HPLC).

The applicant also provided the results of the stability of the NF for one batch at ambient temperature for 8 months in different sets of conditions (darkness and protective atmosphere; light and protective atmosphere; light and open vial). In the open vial only, some change in colour was observed at 4 months, as a result of oxidation. Moisture content also increased, owing to the hygroscopic nature of the product, and purity was slightly reduced.

The Panel considers that the data provided sufficient information with respect to the stability of the NF.

3.1.2. Stability under the intended conditions of use

The stability test of hydroxytyrosol added to vegetable oils at concentrations of 100, 300 and 500 mg/kg reported loss of hydroxytyrosol up to 12% in sunflower oil, 25–33% in olive oil, and 64–72% in soy oil at 40°C, after 12 months. After 4 days at 90°C, the loss of hydroxytyrosol in olive oil (500 mg/kg) ranged from 62% to 99%; whereas, at 180°C, after 1 h the loss of hydroxytyrosol was about 96–99% in olive oils, 94% in soy oil and 54% in sunflower oil. These tests were not performed under 'protecting atmosphere' conditions. The applicant indicated that the NF is not intended to be used at high temperature and thus a warning for not heating oils will be included in the package of the final products.

Upon EFSA's request for additional information on the degradation products of the NF at high temperature, the applicant indicated that no significant differences were reported between the analysis performed with HPLC-UV–MS of an enriched extra virgin olive oil and a natural extra virgin olive oil with similar concentration of hydroxytyrosol after 1 h at 180°C. The formation of decomposition compounds in virgin olive oils suggests the possible formation of dimers and quinones from hydroxytyrosol and other related polyphenols (Antolovich et al., 2004; Di Maio et al., 2011). Thermal oxidation of extra virgin olive oils at 170–220°C causes a significant decrease in hydroxytyrosol- and tyrosol-like substances (Brenes et al., 2002; Attya et al., 2010).

The Panel considers that the data provided sufficient information with respect to the stability of the NF, which however is not expected to be used at high temperature.

3.2. Effect of the production process applied to the NF

The applicant produces the NF by a chemical synthesis which involves several steps (hydrolysis, esterification, reduction and purification). Dihydroxyphenylacetic acid is esterified and the resulting ester is purified, concentrated, and reduced. The crude hydroxytyrosol is extracted and esterified to hydroxytyrosyl acetate, which is then washed with water to eliminate the inorganic salts, hydrolyzed, decolorised and further purified to obtain hydroxytyrosol in high yield and purity.

The Panel considers that the production process is sufficiently described and does not raise concerns about the safety of the NF.

3.3. History of the organism used as a source of the NF

The NF is hydroxytyrosol which is chemically synthesised. Since the NF is not obtained from a biological source, this section is not applicable for this NF.

3.4. Anticipated intake/extent of use of the NF

The applicant intends to add hydroxytyrosol to fish and vegetable oils up to 215 mg/kg and to margarines up to 175 mg/kg (Table 3).

The applicant indicated that the NF is not intended to be used at high temperature and thus a warning for not heating oils will be included in the package of the final products.

Table 3: Proposed uses and maximum use levels of the NF

Proposed uses of the NF	Maximum use levels of the NF
Fish oil Cod liver, fish and herring oils	215 mg/kg
Vegetable oils Almond, coconut, corn, cottonseed, grape seed, linseed oil; oil, frying, blend*; olive oil, palm kernel, palm, peanut, pumpkinseed, rapeseed, safflower, sesame, soybean, sunflower, vegetable, walnut, wheat germ oil	215 mg/kg
Margarines Margarine and similar products; margarine with other ingredients; margarine, low fat and margarine normal fat	175 mg/kg

NF: novel food.
*: NF is not intended to be used at high temperature.

The target group is the general population which excludes children under 36 months of age, pregnant women and breastfeeding women. Foods that are intended for infants and toddlers are excluded from the list of intended food uses of the NF.

3.4.1. Estimate intake of the NF from fortified foods

EFSA performed a detailed assessment for a refined estimate of the anticipated daily intake of the NF at its maximum proposed use levels, using individual data from EU dietary surveys (EFSA, 2011). The lowest and highest mean and 95th percentile anticipated daily intake of the NF (on a mg/kg body weight (bw) basis), among the EU dietary surveys, are presented in Table 4.

Table 4: Refined estimate of anticipated daily intake of the NF: lowest and highest mean and 95th percentile daily intake of the NF among the EU dietary surveys

Population group	Estimate mean intake of the NF across EU dietary surveys (mg/kg bw per day)		Estimate 95th percentile intake of the NF across EU dietary surveys (mg/kg bw per day)	
	Lowest	Highest	Lowest	Highest
Children (3–9 years)	0.03	0.26	0.13	0.48
Adolescents (10–17 years)	0.02	0.15	0.06	0.25
Adults (18–64 years)	0.01	0.12	0.05	0.21
Elderly (above 65 years)	0.01	0.11	0.04	0.24

bw: body weight; NF: novel food.

3.4.2. Intake of hydroxytyrosol from the background diet

The main dietary sources of hydroxytyrosol are olive oils and table olives.

The hydroxytyrosol content in olive oils and table olives is reported in a database on polyphenols in foods (Neveu et al., 2010), which aggregates data from 173 samples of virgin olive oils from 20 publications, 43 samples of extra-virgin olive oils from 8 publications, 31 samples of black olives from 5 publications and 17 samples of green olives from 4 publications. The highest content of free hydroxytyrosol reported in this database is 74.3 mg/kg for olive oils and 4,133 mg/kg for olives. The mean content of free hydroxytyrosol in olive oils and olives as reported in this database is presented in Table 5. The Panel notes that the NF is intended to be added to oils at concentrations of about 3–30 times higher than the high and mean content, respectively, of free hydroxytyrosol in olive oils as reported in this database. However, the Panel notes that in olive oils and olives hydroxytyrosol derives from oleuropein and oleuropein-aglycone.

Table 5: Mean content of free hydroxytyrosol in olive oils and table olives (mg/kg)

Virgin olive oils	3.5
Extra-virgin olive oils	7.7
Black olives	659.3
Green olives	555.7

Based on the reported mean content of free hydroxytyrosol in extra-virgin olive oils (i.e. 7.7 mg/kg) and black olives as reported above (i.e. 659.3 mg/kg) and the consumption of olive oils and olives in the European Union (EU) as reported in the EFSA Comprehensive Food Consumption Database (EFSA, 2011), the daily intake of free hydroxytyrosol from the consumption of olive oils and olives (mean and 95th percentile) is presented Tables 6 and 7. The highest mean and 95th percentile daily intake of free hydroxytyrosol from the consumption of olive oils occur in children (Table 6). The mean daily intake of free hydroxytyrosol from the consumption of olives is in the range of the mean anticipated daily intake of the NF, in all population groups. The 95th percentile daily intake of free hydroxytyrosol from the consumption of olives is in the range of the 95th percentile anticipated daily intake of the NF in adolescents and adults. In children, the range of the 95th percentile daily intake of hydroxytyrosol from the consumption of olives is below the range of the 95th percentile anticipated daily intake for the NF. In elderly, the 95th percentile daily intake of hydroxytyrosol from the consumption of olives is close to the lowest 95th percentile anticipated daily intake for the NF.

The Panel notes that the estimated daily intake of hydroxytyrosol from the consumption of olive oils and olives is based on the reported mean content of free hydroxytyrosol and that higher amount of free hydroxytyrosol in olive oils and olives has been reported (Neveu et al., 2010). The Panel also notes that the hydroxytyrosol content in olive oils and olives concerns free hydroxytyrosol. However, olives contain also the polar polyphenol glucosides oleuropein and ligstroside (Vissers et al., 2004).

During ripening of the olives, oleuropein and ligstroside are enzymatically hydrolysed to their apolar aglycones (by removal of the glucose by β-glucosidase) which are the most abundant phenols in olive oil (up to 460 mg/kg oleuropein-aglycone according to Neveu et al., 2010). Oleuropein- and ligstroside-aglycones are further hydrolysed to hydroxytyrosol and tyrosol, respectively, and elenolic acid (Vissers et al., 2004). Therefore and according to the review on the bioavailability of olive oil phenols in human by Vissers et al. (2004), supported by analytical data by Romani et al. (1999), not only the variety, climate, area of growth, latitude and processing have an impact on the content of these polyphenols and on the ratio between oleuropein, oleuropein-aglycone versus free hydroxytyrosol in olives and olive oils, but also the degree of ripening. The studies by Romani et al. (1999) and Bianco and Uccella (2000), Bianco et al. (2003), which are three of the five publications covered by the polyphenol database (Neveu et al., 2010) used for the calculation of the mean concentration of 659 mg/kg for black olives presented in Table 5, indicate the wide range of ratio between the content of oleuropein, oleuropein-aglycone and free hydroxytyrosol in olives which depends on the variety and the degree of ripeness. Since bioavailability studies on polyphenols from olives and olive oils show that also hydroxytyrosol from oleuropein and oleuropein-aglycone is bioavailable (Section 3.7.1), the Panel considers that the total systemic exposure to hydroxytyrosol from olive oils and olives is higher than the exposure estimated on the basis of the content of free hydroxytyrosol only. On the basis of the mean contents of free hydroxytyrosol, oleuropein and oleuropein-aglycone as reported in the polyphenol database, the total exposure to hydroxytyrosol from olives and olive oils may be estimated to be approximately three and six times, respectively higher than the exposure to only free hydroxytyrosol from these foods.

Table 6: Olive oils consumption and free hydroxytyrosol daily intake from olive oils for consumers-only based on the EFSA Comprehensive European Food Consumption Database

Population group	Olive oils consumption		Free hydroxytyrosol daily intake from the consumption of olive oils	
	Range of means among EU surveys (g/kg bw per day)	Range of 95th percentile among EU surveys[(a)] (g/kg bw per day)	Range of means (mg/kg bw per day)	Range of 95th percentile (mg/kg bw per day)
Children (3–9 years)	0.02–1.09	0.04–2.07	0.00015–0.008	0.0003–0.016
Adolescents (10–17 years)	0.02–0.64	0.06–1.09	0.00015–0.005	0.00046–0.008
Adults (18–64 years)	0.02–0.50	0.06–0.92	0.00015–0.004	0.00046–0.007
Elderly (≥ 65 years)	0.01–0.50	0.21–0.90	0.00007–0.004	0.0016–0.007

bw: body weight.
(a): Based on surveys with at least 60 consumers.

Table 7: Table olives consumption and free hydroxytyrosol daily intake from table olives from consumers-only based on the EFSA Comprehensive European Food Consumption Database

Population group	Table olives consumption		Free hydroxytyrosol daily intake from the consumption of table olives	
	Range of means among EU surveys (g/kg bw per day)	Range of 95th percentile among EU surveys[(a)] (g/kg bw per day)	Range of means (mg/kg bw per day)	Range of 95th percentile (mg/kg bw per day)
Children (3–9 years)	0.03–0.57	0.09–0.41	0.019–0.375	0.059–0.270
Adolescents (10–17 years)	0.02–0.31	0.09–0.58	0.013–0.204	0.059–0.382
Adults (18–64 years)	0.03–0.28	0.12–0.63	0.019–0.185	0.079–0.415
Elderly (≥ 65 years)	0.02–0.19	0.09	0.013–0.125	0.059

bw: body weight.
(a): Based on surveys with at least 60 consumers.

3.4.3. Intake of hydroxytyrosol from food supplements

Following a comment from a Member State on the combined intake of hydroxytyrosol from food supplements, the applicant noted that there are food supplements on the market with 25 mg per serving.

3.5. Nutritional information on the NF

The addition of the NF to oils and fats does not change the inherent nutritional value of these foods.

The Panel considers that the NF is not nutritionally disadvantageous.

3.6. Microbiological information on the NF

Analysis of four non-consecutive batches showed the absence of microorganisms (cfu/g < 10) including aerobic mesophiles, total enterobacteriaceae, *E. coli*, and moulds and yeasts.

The Panel considers that the microbiological information provided does not raise safety concerns.

3.7. Toxicological information on the NF

3.7.1. Absorption, distribution, metabolism and excretion (ADME)

Animal studies

The applicant provided four animal studies on ADME of chemically synthesised hydroxytyrosol (Bai et al., 1998; D'Angelo et al., 2001; Tuck et al., 2001; Seprox BIOTECH SL, 2013).

The plasma kinetics of hydroxytyrosol (95% purity produced by the applicant) was studied in rats (Seprox BIOTECH SL, 2013). Groups of 18 Sprague–Dawley rats (n = 9 males and 9 females) were given the test items by gavage at single doses of 1 or 5 mg/kg bw. The control group (n = 3 males and 3 females) was administered the vehicle (5% ethanol in water). Blood samples (n = 3 males and 3 females) were taken from the test groups at 0.5, 1, 2, 4, 8 and 24 h after administration, and from the control group once before and 2 h after dosing. Plasma hydroxytyrosol was analysed using LC–MS/MS and kinetic parameters (c_{max}, t_{max} and area under the curve (AUC_{0-24})) were calculated. The levels in urine collected over a period of 24 h were also determined. Kinetic parameters for hydroxytyrosol could not be calculated as the levels in plasma were hardly over the lower limit of quantification. However, the study suggests a quick conversion of hydroxytyrosol into 3,4-dihydroxyphenylacetic acid (DOPAC) after oral administration. The main compound excreted in the 24 h urine was hydroxytyrosol acetate. The amount of hydroxytyrosol acetate excreted in urine was higher in females than in males.

Bai et al. (1998) determined hydroxytyrosol plasma levels in male Wistar rats after oral administration of 55 mg/kg bw of hydroxytyrosol, after overnight fasting. Plasma levels (n = 3 rats per group) were quantified at various time points until 40 h after administration using a GC–MS selected ion monitoring method. Hydroxytyrosol was measurable at the first time point, i.e. 2 min after administration, individual levels were highest at 5 or 10 min, decreased rapidly to a certain level within approximately 60 min and then slowly for 1–2 h. Hydroxytyrosol was thus rapidly absorbed and almost completely eliminated from the blood after 180 min.

Tuck et al. (2001) investigated the fate of radiolabelled hydroxytyrosol (and tyrosol) in male Sprague–Dawley rats. Following a single intravenous (i.v.) injection (in saline solution) or oral administration by gavage (in olive oil or aqueous solution) of chemically synthesised ^{3}H-labelled hydroxytyrosol (^{3}H label on the aromatic ring) to groups consisting of five animals each, urine was collected after 1, 2, 3, 4, 8 and 24 h. Total radioactivity in each sample was determined by liquid scintillation counting. In addition, samples treated or not treated with β-glucuronidase or sulfatase were analysed for the presence of metabolites and titrated water using HPLC coupled with radiometric detection. Within 24 h after dosing approximately 95% (oral in oil and i.v.) and 70% (oral in water) of the radiolabel, corresponding to hydroxytyrosol and metabolites, was excreted in urine. The majority was eliminated within 2 h after i.v. dosing and 4 h after oral dosing. The estimated bioavailability of hydroxytyrosol when given orally in oil and water was 99% and 75%, respectively. There was no significant difference in the amount of hydroxytyrosol (plus metabolites) eliminated in urine within 24 h between the group orally administered the oil-based solution and the group dosed intravenously.

D'Angelo et al. (2001) investigated the metabolism of hydroxytyrosol in 12 male Sprague–Dawley rats following a single intravenous administration of ^{14}C-labelled chemically synthesised hydroxytyrosol

(1.5 mg/kg bw). Total radioactivity was measured in blood (whole blood and plasma) and tissue samples (liver, kidneys, lungs, skeletal muscle, heart and brain) and gastrointestinal content obtained from two rats each at different time points (5, 10, 30, 60, 120 and 300 min after injection) as well as in urine and faeces collected up to 300 min after injection. Less than 8% of the injected radioactivity was present in the blood 5 min after injection, approximately 4% after 10 min and only 0.1% after 300 min. The accurate half-life in blood could not be determined but was estimated to be within minutes. Regarding the analysed tissues, the amount of radioactivity was highest 5 min after injection followed by a gradual decrease. The radioactivity in the kidneys was about 10 times higher than in the other organs. Ninety per cent of the injected dose was excreted in urine collected up to 5 h after administration, indicating that renal excretion of hydroxytyrosol and/or its metabolites is the major elimination route. About 9% of the radioactivity was found in the gastrointestinal content 5 min after administration, decreasing to 2.5% at 5 h after administration. In all investigated tissues as well as in plasma four oxidised and/or methylated derivatives (i.e. homovanillic alcohol, homovanillic acid, DOPAC and 3,4-dihydroxyphenylacetaldehyde) were present already 5 min after injection. A significant fraction of total radioactivity was associated with sulfate-conjugated forms, which also represent the major urinary excretion products. On the basis of the results, the authors proposed a metabolic pathway of hydroxytyrosol, implying the involvement of catechol-O-methyltransferase, alcohol dehydrogenase, aldehyde dehydrogenase and phenol sulfotransferase.

Overall, the data provided on ADME in rats indicate that synthetic hydroxytyrosol is highly bioavailable, quickly absorbed with a c_{max} reached within few minutes, rapidly oxidised/conjugated and eliminated predominantly via the kidneys within a few hours.

Human studies

Gonzalez-Santiago et al. (2010) studied the absorption of highly purified hydroxytyrosol (99.5%) obtained from olive mill waste water extract and administered to 10 subjects at a single oral dose of 2.5 mg/kg bw as powder in an aqueous solution after an overnight fast lasting of at least 10 h. After administration, the plasma concentrations of free hydroxytyrosol and its metabolite homovanillic alcohol increased in all subjects. The kinetic data calculated from the absorption curves showed large inter-individual differences in the absorption profile. The maximum concentration in plasma (c_{max}) for hydroxytyrosol and homovanillic alcohol was reached at 13 and 16.7 min (t_{max}), respectively. Following a rapid decline, undetectable levels were reached 1 h after administration. The estimated half-life for both substances was approximately 8 min. The bioavailability of free hydroxytyrosol calculated from the AUC was 6.2% (range 2.4–11.8%). The main metabolites found in the 24 h urine were homovanillic acid (31% in the free form, 11% as sulfate derivative and 10% as glucuronide derivative) and DOPAC (23% as glucuronide derivative, 13% in the free form and 6% as sulfate derivative); whereas only 5% was detected as hydroxytyrosol (3.8% as sulfate derivative and 1.2% as glucuronide derivative).

The Panel notes the relatively low bioavailability of hydroxytyrosol in this study when consumed in an aqueous solution and a higher bioavailability of hydroxytyrosol when administered in oil, as reported in the rat study by Tuck et al. (2001). A study by Visioli et al. (2003) on the bioavailability of hydroxytyrosol and oleuropein-aglycone showed that the percentage of excreted hydroxytyrosol by humans was significantly higher when consumed with olive oil in comparison to yogurt. This can be ascribed to the higher solubility of the aglycone in oil and proves that hydroxytyrosol derived from oleuropein-aglycone is bioavailable.

In addition, some human studies reported on the bioavailability and plasma kinetics of hydroxytyrosol from olive oils, olives and extracts from natural sources. Although the Panel considers that the test materials used in these studies are not representative of the NF, some of these studies provide information on the plasma kinetics of hydroxytyrosol from the consumption of olive oils and olives and show the systemic exposure to hydroxytyrosol from oleuropein and oleuropein-aglycone contained in olive oils or olive extracts. These studies have been reviewed by Vissers et al. (2004), de la Torre (2008) and Fito et al. (2007).

Vissers et al. (2004) summarised the reported recovery of ingested olive oil phenols as tyrosol and hydroxytyrosol in urine in humans ranging between 5% and 72%, most of them conjugated to glucuronic acid according to Visioli et al. (2000a,b), Miro-Casas et al. (2001a,b) and Vissers et al. (2002). This wide range is probably due to the various approaches to calculate urinary excretion and to different analytical analyses.

In the review on the bioavailability of olive oil phenolic compounds in humans, de la Torre (2008) concluded that hydroxytyrosol from olive oil is well absorbed in the gastrointestinal tract and

undergoes a first pass metabolism both in gut and liver, which leads to the formation of sulfate and glucuronide conjugates. In body fluids, the free form is therefore almost undetectable. It was noted that hydroxytyrosol is also a dopamine metabolite and body fluids contain hydroxytyrosol from exogenous and endogenous sources.

On the basis of three studies by Miro-Casas et al. (2001a,b, 2003), also Fito et al. (2007) considered in their review that around 98% of tyrosol and hydroxytyrosol are present in plasma and urine as mainly glucuronides and to a lesser degree to sulfates, which suggests an extensive first pass effect of the ingested primary forms in the intestine/liver. Regarding bioavailability, Fito et al. (2007) referred to the placebo-controlled study by Vissers et al. (2002) who investigated the bioavailability of three different olive-derived polyphenols consumed as supplements by ileostomy subjects without gastrointestinal disease: (1) a non-polar extract from extra-virgin olive oil containing about 68% of the ligstroside- and oleuropein-aglycones, about 30% tyrosol and only a small amount of hydroxytyrosol (total polyphenol intake: 371 μmol), (2) polar polyphenols extracted from extra virgin oil consisting of more than 70% of tyrosol and hydroxytyrosol and less than 30% of ligstroside- and oleuropein-aglycones (total polyphenol intake: 498 μmol), and (3) a supplement containing only oleuropein-glucoside (total polyphenol intake: 190 μmol). The ingested dose of free hydroxytyrosol from these three food supplements was 3, 198 and 0 μmol. However, the excretion in ileostomy effluent over 24 h was quite similarly small for the three groups, i.e. 4.4, 1.8 and 2.2 μmol, respectively. The total excretion of the ingested olive polyphenols in the ileostomy effluent was less than 45%, 32% and 34%, respectively. The authors therefore estimated that at least 55% of the ingested olive polyphenols from each of the three supplements were bioavailable. The urinary excretion of hydroxytyrosol over 24 h was 10.8, 24.7 and 28.4 μmol, respectively. The authors suggested that oleuropein-glucoside and oleuropein-aglycone are hydrolysed either in the gastrointestinal tract or in the circulation after absorption.

Kountouri et al. (2007) investigated the bioavailability of hydroxytyrosol from the consumption of 100 mg of olives, which contained 76.73 mg of hydroxytyrosol and 19.48 mg of tyrosol, in healthy males (n = 7), following a 3-day wash-out period on a polyphenol-free diet. The amount of oleuropein in olives was below the limit of quantification. Plasma levels of hydroxytyrosol, tyrosol, homovanillic acid, homovanillic alcohol and DOPAC increased after consumption of olives as compared to baseline (wash-out period), with maximum levels reached after 1 h. Plasma polyphenols mainly occurred as glucuronide derivatives. Hydroxytyrosol was mainly excreted in urines in the form of homovanillic acid, DOPAC and homovanillic alcohol. Excretion rates of hydroxytyrosol, tyrosol and its metabolites were maxima at 0–4 h after consumption of olives. On the basis of plasma levels and excretion rates, the study reported that hydroxytyrosol possesses good bioavailability, although no quantitative estimate on the bioavailability hydroxytyrosol was provided in this study.

Another study on the polyphenol content in table olives reported 86.3% 'bioaccessibility' for hydroxytyrosol, which suggests a high degree of bioavailability of hydroxytyrosol (D'Antuono et al., 2016). 'Bioaccessibility' was defined as the percentage of the amount of hydroxytyrosol in the supernatant of homogenised and *in vitro* digested olives (treated with human digestive fluids) out of the amount of hydroxytyrosol in the undigested table olives.

The data above from human studies indicate that the systemic exposure to hydroxytyrosol results not only from the intake of free hydroxytyrosol, but to a significant degree also from ingested oleuropein and its aglycone contained in olives and olive oils. The Panel considers that these studies indicate that hydroxytyrosol derived from its natural sources is bioavailable for humans, metabolised and rapidly eliminated primarily in the urine as glucuronide and sulfate derivatives.

Overall, the provided data on the kinetics suggest that in both rats and humans hydroxytyrosol is quickly absorbed, has a half-life time of a few minutes and is eliminated by the kidneys as either free hydroxytyrosol, or in oxidised/conjugated forms (glucuronide and sulfate derivatives).

3.7.2. Genotoxicity

The potential genotoxicity of hydroxytyrosol was investigated in a bacterial reverse mutation test and an *in vitro* chromosome aberration test using human lymphocytes (Auñon-Calles et al., 2013b; study report by Vivotecnia Research, 2010 and by Harlan CCR, 2013), which were conducted with the NF. These studies were conducted in compliance with test guidelines No 471 and No 473, respectively, from the Organisation for Economic Co-operation and Development (OECD) (OECD, 1997a,b) and principles of Good Laboratory Practice (GLP) (OECD, 1998b; European Directive 2004/10/EC).

In the bacterial reverse mutation test, *Salmonella* Typhimurium strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* strain WP2 (pKM101) were exposed to hydroxytyrosol (purity 99.59%), at concentrations up to 5.00 μL/plate, in the presence or absence of metabolic activation (S9-mix), using the plate-incorporation and the pre-incubation methods. No cytotoxic activity was observed up to the highest concentration tested. This study showed no mutagenicity of hydroxytyrosol, with or without metabolic activation, up to the highest concentration tested of 5.00 μL/plate.

In the *in vitro* chromosome aberration test, human lymphocytes were exposed to hydroxytyrosol (purity 99.31%), at concentrations up to 1,542 μg/mL (~ 10 mM), for 4 h in the presence or absence of S9-mix. No visible precipitation of the test item in the culture medium was noted, with or without S9-mix, and no relevant influence on osmolarity or pH value were observed. Concentrations of 881.1 μg/mL and higher were toxic to the cells, thus concentrations of 164.4, 287.7 and 503.5 μg/mL were evaluated. At these concentrations, no evidence of an increase in polyploidy was noted. This study reported a statistically significant increase in the number of cells with structural chromosome aberrations in the absence (9% excluding gaps at 503.5 μg/mL) and presence of S9-mix (3.5% excluding gaps at 287.7 μg/mL and 4.5% at 503.5 μg/mL). The values exceeded the range of the historical solvent control data of the laboratory (0.0–3.0% aberrant cells, excluding gaps). Therefore, hydroxytyrosol was considered clastogenic and no further tests for longer treatment periods were conducted.

According to the EFSA scientific opinion on genotoxicity testing strategies, an appropriate *in vivo* study may be required to assess whether the genotoxic potential observed *in vitro* is expressed *in vivo* (EFSA Scientific Committee, 2001). Thus, the applicant provided a publication on a mammalian bone marrow chromosome aberration test with hydroxytyrosol using Wistar rats (Dolan et al., 2014). Based on additional information from the applicant, which was provided upon request by EFSA, the Panel considers that the test material used in this study is representative of the NF and appropriate for the safety testing. The study was conducted in accordance with the OECD test guideline No 475 (OECD, 2014). Hydroxytyrosol was administered by gavage to two groups of rats (n = 5 per sex) at a dose of 2,000 mg/kg bw (one group for the 24 h and one for the 48-h evaluation time point). Two groups (n = 5 per sex) were administered the vehicle (distilled water) and served as negative controls. The positive control group (n = 5 per sex) was administered cyclophosphamide by intraperitoneal injection. The publication reported signs of acute toxicity after administration of hydroxytyrosol in some but not all animals. There was no statistically significant increase in the number of cells with chromosome aberrations (excluding gaps) in the hydroxytyrosol-treated groups when compared with the negative control groups. According to the authors, the mean values of aberrant cells in all groups were within the historical range for negative control values for the laboratory. The mitotic index values in the test groups were not different from the values for the concurrent negative controls. The positive control cyclophosphamide induced the expected increase in the number of cells with chromosome aberrations and a decrease in the mitotic index. Based on these results, the Panel concludes that hydroxytyrosol at the oral limit dose of 2,000 mg/kg bw did not induce chromosome aberrations in bone marrow cells of the rat and is not a clastogen *in vivo*. The Panel notes that it has not been demonstrated that the test material reached the bone marrow. However, the Panel acknowledges the high-dose of the material which has been tested in this *in vivo* study.

In order to further address the potential genotoxicity of the NF, the applicant also provided a publication on genotoxicity tests with a hydrolysed aqueous olive pulp extract, containing 2.4% of hydroxytyrosol (Christian et al., 2004). The Panel considers that this test material is not representative of the NF and thus no conclusions with regard to the safety of the NF can be drawn from this study.

The applicant also referred to the publication of Kirkland et al. (2015) who performed among other studies genotoxicity tests with various olive extracts containing up to 40% hydroxytyrosol. In micronucleus assays *in vitro*, extracts containing 15% (+/– S9-mix) and 40% hydroxytyrosol (–S9-mix) as well as pure hydroxytyrosol (–S9-mix) increased the number of cells with micronuclei. The increases only occurred at concentrations where cytotoxicity (> 50 or 60%) was observed. Therefore, the authors considered that they may be an indirect consequence of excessive toxicity. Nevertheless, additional *in vivo* studies were carried out to further investigate the unclear results in the previous studies. In an *in vivo* micronucleus study, two different extracts containing 40% hydroxytyrosol were administered to male Wistar rats. One of the extracts was prepared using a process avoiding any high temperature processing steps. Groups of animals (n = 7) were administered the extracts by gavage at dose levels corresponding to 0 (vehicle control), 500, 1,000 and 2,000 mg hydroxytyrosol/kg bw on a single occasion. Animals were sacrificed at 24 h (all dose groups and positive control group with cyclophosphamide) and at 48 h (2,000 mg/kg bw group and vehicle control group) and bone marrow

cells were collected for analysis of micronuclei. The frequency of micronuclei in polychromatic erythrocytes (PCE) was determined from scoring 6,000 PCEs per animal. Cytotoxicity was determined as the number of PCEs per 2,000 erythrocytes. Blood samples were taken 30 min after dosing from high dose and vehicle control animals for analysis of hydroxytyrosol in plasma. Statistically significant increases in micronucleus frequencies compared with the vehicle control group were seen in the low and mid dose groups (3 of 4 values). The increases were small, fell well within the historical control ranges, and were not dose-related. No statistically significant differences were seen in the 2,000 mg/kg bw group either at 24 or 48 h. The positive control cyclophosphamide induced the expected significant increase. The ratio of PCEs to total erythrocytes was not changed, but plasma analysis showed that free and total hydroxytyrosol were present (20 and 120 μg/mL, respectively) and therefore the bone marrow was exposed to hydroxytyrosol. Furthermore, in the 90-day repeated dose oral toxicity study, findings in the haematology indicate that the bone marrow was exposed to hydroxytyrosol.

Based on the studies described above, the Panel considers that the clastogenicity of hydroxytyrosol observed *in vitro* is not expressed *in vivo*. The Panel concludes that there is no concern with regard to potential genotoxicity of the NF.

3.7.3. Acute toxicity studies

In an acute oral toxicity study by D'Angelo et al. (2001) Sprague–Dawley rats (n = 6 males and n = 6 females) were administered by oral gavage a single dose of 2,000 mg/kg bw of chemically synthesised hydroxytyrosol (purity 98.9%). Rats were observed and weighed daily for 2 weeks. During the study period, no death occurred and the only clinical sign observed in males and females was piloerection, which started 2 h after treatment and disappeared within 48 h from treatment. On day 14, the rats were sacrificed and gross pathological changes in main organs were evaluated. The authors concluded that there were no adverse effects after administration of hydroxytyrosol at a dose of 2,000 mg/kg bw.

3.7.4. Repeated dose toxicity studies

A publication on a 90-day repeated dose toxicity study with a hydrolysed aqueous olive pulp extract, containing 2.4% of hydroxytyrosol (Christian et al., 2004) was provided by the applicant. The Panel considers that this test material is not representative of the NF and thus no conclusions with regard to the safety of the NF can be drawn from this study.

Synthetic hydroxytyrosol was tested in a subchronic 90-day oral toxicity study in rats (Auñon-Calles et al., 2013a; study report by Harlan Laboratories SA, 2013). The study was conducted in accordance with OECD guideline No 408 and in compliance with GLP (OECD, 1998a,b). Hydroxytyrosol (purity 99.28%) dissolved in water was administered by gavage to groups of rats (n = 10 per sex) (Wistar Hannover RccHan™:WIST) at dose levels of 0 (vehicle control), 5, 50 or 500 mg/kg bw per day for 91–92 consecutive days. Five additional animals per sex were allocated to the control group and the high-dose group in order to assess the reversibility or progression of any test item-related changes after a 4-week treatment-free recovery period.

Table 8 summarises the findings from this subchronic 90-day toxicity study.

During the treatment period, there was no mortality. Salivation was recorded before and/or after the administration in all animals from the high-dose group and occasionally in the mid- and low-dose groups. According to the authors, this effect is attributable to the bitter taste of hydroxytyrosol and/or the physical characteristics of the formulation (slightly oily and dense). Apart from salivation no treatment-related clinical signs were noted.

Table 8: Summary of the findings from the subchronic (90-day) toxicity study with the NF

	5 mg/kg bw per day	50 mg/kg bw per day	500 mg/kg bw per day	4 week recovery
Clinical signs	Salivation (occasionally in one f)	Salivation (occasionally four m and two f)	Salivation (all f and m before and after administration from day 3 until the end of treatment)	
Functional tests	Motor activity ↑ m (20 min) Forelimb grip strength ↓ (f)	Motor activity ↑ m (10/20 min) Scabs and desquamation in the sacral region, scabs at the base of the tail and hair loss at the head (m)	Motor activity ↓ m (30/40/50/60 min) ↑ f (60 min)	Motor activity ↑ f (60 min)
Body weight			↓ m (8% at day 91, but not statistically significant different) bw gains ↓ (m) but only statistically significant at day 91 (14%) bw gains ↓ (f) but statistically significant only at weeks 2 and 3 (38%) and at weeks 4 and 5 (20%)	
Haematology	m Reti rel. ↑ (not abs.) (18%)	m Monocyte rel. ↑ (not abs.) (33%) f MCV ↑ (3%), MCH ↑ (4%)	m HDW ↓ (8%) f MCV ↑ (4%), MCH ↑ (4%), HFR ↑ (62%) WBC ↑ (30%), EOS abs. ↑ (10%)	m HDW ↓ (12%), RDW % ↓ (10%) f HDW ↓ (6%) MCHC ↓ (2.6%) PLT ↑ (26%) PT ↑ (6%)
Clinical chemistry	m ASAT ↑ (46%)	m ASAT ↑ (47%) Ca ↑ (7%), K ↓ (10%)	m Gluc ↓ (11%), (ASAT ↑not statistically significant 26%), Alb. ↑ (7%) Creat. ↓ (24%), Ca ↑ (7%)	m ASAT ↑ (42%)
Organ weights	m ↑ rel. testes (11%)	m ↑ rel. heart (7%), testes (10%)	m ↑ rel. brain (12%), heart (14%), kidneys (19%), testes (15%), epididymis (19%), mandibular salivary glands (18%) f ↑ rel. heart (12%), liver (13%), kidneys (13%) mandibular salivary glands (10%) ↑ rel. kidneys (12%) (to brain wt)	m ↑ abs. testes (19%) f ↑ abs. liver (17%), kidneys (15%) ↑ (rel liver (13%) not stat. sign., kidneys (11%) not stat. sign.) ↑ rel. liver (18%), kidneys (15%) (to brain wt))

Abs.: absolute; Alb.: albumin; ASAT: aspartate aminotransferase; Creat: creatinine; f: females; HCT: haematocrit; HFR: reticulocyte maturity index; HDW: haemoglobin concentration distribution width; m: males; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration, MCV: mean corpuscular volume; PLT: platelet (thrombocyte) count; PT: prothrombin time; RDW: red cell volume distribution width; rel.: relative.

A limited number of functional tests showed a statistically significant difference in the hydroxytyrosol-treated groups as compared with the control group: lower locomotor activity in males of the high-dose group (during four 10-min intervals of a 60-min observation period); higher values of locomotor activity were occasionally noted in males of the mid (during two intervals) and low (during one interval) dose groups and females of the high dose group (during one interval); lower forelimb grip strength in low-dose group female. Ophthalmoscopic examinations showed no ocular alterations.

Although not statistically significant, males in the high-dose group showed lower body weights throughout the treatment period as compared with the control group (approximately 8% lower body weight after 13 weeks). After the 4-week recovery period, male body weights were nearly equalised. Body weight gains in high-dose males were also lower as compared to control, but the difference reached statistical significance at day 91 only. High-dose females occasionally showed a statistically significant lower body weight gain during the first 5 weeks, but no relevant differences in mean body weights were noted. There were no significant differences in feed consumption.

Haematology showed several statistically significant differences in the hydroxytyrosol-treated groups as compared with the control group (i.e. in males: lower haemoglobin distribution width (HDW) in the 500 mg/kg bw group, higher relative monocyte count in the 50 mg/kg bw group, and higher relative reticulocyte count in the 5 mg/kg bw group; in females: higher mean cell volume (MCV) and mean cell haemoglobin (MCH) in the 50 and 500 mg/kg bw groups; higher reticulocytes with high fluorescence (HFR), higher white blood cell count (WBC) and higher absolute eosinophil count (EOS) in the 500 mg/kg bw group). At the end of the recovery period, HDW was significantly lower in males and females; red cell distribution width (RDW) was lower in males; in females mean corpuscular haemoglobin concentration (MCHC) was lower, platelet count (PLT) was higher and prothrombin time (PT) was prolonged.

Clinical chemistry analysis showed statistically significant differences in males: lower glucose and creatinine levels and higher albumin level in the 500 mg/kg bw group; higher calcium levels in the 50 and 500 mg/kg bw groups; higher aspartate aminotransferase (ASAT) values in the 5 and 50 mg/kg bw groups (higher mean value but not statistically significant in the 500 mg/kg bw group); lower potassium level in the 5 mg/kg bw group. At the end of the recovery period, ASAT value in males in the 500 mg/kg bw group was still significantly higher compared with the control group. No statistically significant differences in clinical chemistry parameters were observed in females at the end of the treatment and at the end of the recovery period.

Urinalysis did not reveal statistically significant differences compared with the control group.

Macroscopic findings recorded at necropsy are not considered treatment-related.

Organ weight determinations for high-dose males showed statistically significant higher relative brain, heart (also in the mid-dose group), kidneys, testes (also in the low- and mid-dose groups), epididymis and mandibular salivary gland weights. Absolute organ weights in males did not differ significantly compared with the controls. High-dose females showed no statistically significant changes in absolute organ weights but significantly higher relative heart, liver, kidneys (also in relation to brain weight) and mandibular salivary gland weights. At the end of the recovery period, statistically significant higher absolute testes weight was noted in males, and higher liver and kidneys weights (both absolute and in relation to brain weight) in females. Microscopic examination of tissues showed no relevant differences in the incidence and severity of histopathological findings. The findings can be considered as spontaneous and typical for rats of this strain and age.

This 90-day oral toxicity study reported statistically significant higher organ weights in males and females in the 500 mg/kg bw group (e.g. relative kidneys weights both in males and females; relative liver weights in females; relative testes and epididymis weights in males). After the 4-week recovery period, some organ weights were reported to be still higher in the 500 mg/kg bw group as compared to the control group (e.g. statistically significant higher absolute liver and kidneys weights in females; higher (but not statistically significant) relative liver and kidneys weights in females). The Panel notes the statistically significant reduction in body weight gain in males in the high-dose group at day 91, which is reflected by lower body weights in the high-dose group as compared to the control group. Based on these observations, as well as other findings in the high-dose group (e.g. increases in white blood cell and eosinophil counts in females), the Panel considers that the no observed adverse effect level (NOAEL) of this study is 50 mg/kg bw per day of hydroxytyrosol.

The Panel also notes that similar changes in organ and body weights were reported in a subchronic oral toxicity study in which hydroxytyrosol (at dose levels of 125, 250 and 500 mg/kg bw per day) was administered as a constituent of an olive extract by gavage to Wistar rats for 90 days (Heilman et al., 2015). The olive extract tested contained 36.2% hydroxytyrosol and 66.6% total phenols. According to

the authors, this study was conducted in accordance with OECD guideline No 408 and in compliance with GLP (OECD, 1998a,b). During the treatment period, males in the high-dose group showed lower body weights when compared with the control group; the difference reached statistically significance during weeks 6–10. At week 13, the difference in body weight and body weight gain as compared to the control group was approximately 9% and 17%, respectively. At the end of the 4-week recovery period, the body weight tended to equalise. Statistically significant higher relative liver, heart and kidneys weights were reported in males and females in the high-dose groups as compared to control group. After the 4-week recovery period, relative organ weights were still reported to be higher. Based on the reduction in body weight and body weight gain in the high-dose males, the authors concluded that the lowest observed adverse effect level (LOAEL) was 500 mg/kg bw per day of hydroxytyrosol and the NOAEL in this study was 250 mg/kg bw per day of hydroxytyrosol.

3.7.5. Developmental and reproductive toxicity studies

The applicant provided a publication on a reproductive toxicity and teratogenicity study in rats with a hydrolysed aqueous olive pulp extract, containing 2.4% of hydroxytyrosol (Christian et al., 2004). The Panel considers that this test material is not representative of the NF and thus no conclusions with regard to the safety of the NF can be drawn from this study.

3.7.6. Human studies

The applicant referred to human intervention studies on the effect of olive oils with different phenolic content or phenolic extracts from natural sources on blood parameters and biomarkers of oxidative damage (Bitler et al., 2007; Raederstorff, 2009; de Bock et al., 2013). The Panel considers that the test materials are not representative of the NF and thus no conclusions can be drawn from these studies on the safety of the NF.

3.8. Allergenicity

The applicant presented the results on residual protein levels, which ranged between 0.035% and 0.045% (colorimetric Bradford assay method), for four batches of the NF.

In reply to an EFSA's request on protein residues, the applicant provided a description of the filtering steps to be introduced in the manufacturing process in order to reduce the protein residues in the final product.

Based on the data provided, the Panel considers that the likelihood of allergic reactions to the NF is low.

4. Discussion

The NF subject of this application is hydroxytyrosol, which is chemically synthesised. Hydroxytyrosol is naturally present in foods, mainly in olive oils and in table olives. The information provided on the composition, specifications, batch-to-batch variability, stability and production process of the NF is sufficient and does not raise concerns about the safety of the NF.

The applicant intends to add hydroxytyrosol to fish and vegetable oils up to 215 mg/kg and to margarines up to 175 mg/kg. The target group is the general population which excludes children under 36 months of age, pregnant women and breastfeeding women. The NF is not intended to be used at high temperature, and thus, the applicant indicated that a warning for not heating oils will be included in the package of the final products.

Based on individual data from EU dietary surveys and the proposed maximum use levels of the NF, the anticipated daily intake of the NF for each population group (children, adolescents, adults and elderly) has been calculated. The main dietary sources of hydroxytyrosol are olive oils and table olives. The daily intake of hydroxytyrosol from the consumption of olive oils and olives has been calculated based on the mean content of free hydroxytyrosol in olive oils (7.7 mg/kg) and in olives (659.3 mg/kg). The Panel notes that higher amount of free hydroxytyrosol in olive oils and olives has been reported and that the NF is intended to be added to oils at concentrations of about 3–30 times higher than the high (4,133 mg/kg) and mean (7.7 mg/kg) content, respectively, of free hydroxytyrosol in olive oils.

The Panel also notes that the content of hydroxytyrosol in olive oils and olives concerns free hydroxytyrosol. However, in olive oils and olives, hydroxytyrosol derives from oleuropein and oleuropein-aglycone. A wide range of content of free hydroxytyrosol, oleuropein and oleuropein-aglycone, which depends on the variety and degree of ripeness of olives, has been reported for olives and olive oils.

Since bioavailability studies on polyphenols from olives and olive oils show that also hydroxytyrosol from oleuropein and oleuropein-aglycone is bioavailable, the Panel considers that the total systemic exposure to hydroxytyrosol from olive oils and olives is higher than the exposure estimated on the basis of the content of free hydroxytyrosol only (approximately six and three times higher for olive oils and olives, respectively, based on a mean content of oleuropein and oleuropein-aglycone).

Overall, the provided data on the kinetics suggest that in both rats and humans hydroxytyrosol is quickly absorbed, has a half-life time of a few minutes and is eliminated by the kidneys as either free hydroxytyrosol, or in oxidised or conjugated forms (glucuronide and sulfate derivatives).

Taking into account the intended use levels, the Panel considers that the consumption of the NF is not nutritionally disadvantageous.

Based on the studies provided, the Panel concludes that there is no concern with regard to potential genotoxicity of the NF. The NF was tested at dose levels of 5, 50, or 500 mg/kg bw per day in a subchronic 90-day oral toxicity study in rats. Based on changes in body and organ weights in the highest dose group tested in this study, the Panel considers as the NOAEL the dose of 50 mg/kg bw per day. The findings in this 90-day study with the NF were supported by the observations in another 90-day oral toxicity study in which hydroxytyrosol (at dose levels of 125, 250 and 500 mg/kg bw per day) was administered as a constituent of an olive extract to rats for 90 days.

Considering the NOAEL of 50 mg/kg bw per day in the subchronic toxicity study with the NF and the maximum anticipated daily intake for the NF, the margin of exposure (MoE; i.e. the ratio between the NOAEL and the maximum anticipated daily intake of the NF) would result in 100 for children (3–9 years of age) and at least 200 for adolescents, adults (excluding pregnant and breastfeeding women) and elderly.

Taking into account that the anticipated daily intake of the NF would be in the range of or even less than the exposure of hydroxytyrosol from the consumption of olive oils and olives, which has not been associated with adverse effects, and considering the similar kinetics of hydroxytyrosol in rats and humans, the Panel considers that the MoE for the NF at the intended uses and use levels is sufficient for the target population.

5. Conclusions

The Panel concludes that the novel food, hydroxytyrosol, is safe under the proposed uses and use levels.

Steps taken by EFSA

1) Letter from the European Commission to the European Food Safety Authority with the request for a scientific opinion on the safety of 'hydroxytyrosol' as a novel food ingredient. SANTE/E6/SS/ks D (2015) 5373931; Ref. Ares(2015)5211344, dated 19 November 2015
2) On 26 November 2015, EFSA received the following documentation: dossier 'hydroxytyrosol', which was submitted by Seprox Biotech; initial assessment report carried out by the Food Safety Authority of Spain: 'Report of the Scientific Committee of the Spanish Agency for Consumer Affairs, Food Safety and Nutrition (Agencia Española de Consumo, Seguridad Alimentaria y Nutrición, AECOSAN) on a request for initial assessment for marketing of synthetic hydroxytyrosol under Regulation (EC) No 258/97 concerning novel foods and novel food ingredients'; Member States' comments and objections; response by the applicant to the initial assessment report.
3) On 7 January 2016, EFSA sent a request to the applicant to provide missing information to accompany the application.
4) On 28 January 2016, EFSA received the missing information as submitted by the applicant. After checking the content of the full dossier, including the missing information, EFSA considered the application valid as of 04 February 2016.
5) On 2 May, 1 June and 19 October 2016, EFSA sent requests to the applicant to provide additional information to accompany the application.
6) Additional data were provided by the applicant on 4 July, 5 and 11 August, 4 October, 29 November 2016 and 13 January 2017.
7) During its meeting on 31 January 2017, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of hydroxytyrosol as a novel food pursuant to Regulation (EC) No 258/97.

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Abbreviations

^{1}H-NMR	proton nuclear magnetic resonance
^{13}C-NMR	carbon-13 nuclear magnetic resonance
ADME	absorption, distribution, metabolism and excretion
Alb.	albumin
ASAT	aspartate aminotransferase
AUC	area under the curve
bw	body weight
cfu	colony-forming units
Creat.	creatinine
DOPAC	3,4-dihydroxyphenylacetic acid
DOPET	3,4-hydroxyphenylethanol
EOS	eosinophil count
GC	gas chromatography
GLP	good laboratory practice

HCT	haematocrit
HDW	haemoglobin distribution width
HFR	reticulocyte maturity index
HMA	halogen moisture analyser
HPLC	high-performance liquid chromatography
IC	ion-exchange chromatography
ICP-MS	inductively coupled plasma mass spectrometry
IUPAC	International Union of Pure and Applied Chemistry
i.v.	intravenous
LC–MS	liquid chromatography–mass spectrometry
LC–MS/MS	liquid chromatography–tandem mass spectrometry
LOAEL	lowest observed adverse effect level
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
MoE	margin of exposure
MS	mass spectrometry
NF	novel food
NOAEL	no observed adverse effect level
OECD	organisation for economic co-operation and development
PCE	polychromatic erythrocytes
PLT	platelet (thrombocyte) count
PT	prothrombin time
RDW	red cell volume distribution width
UV	ultraviolet
WBC	white blood cell count