Safety of Cetylated Fatty Acids as a Novel Food pursuant to Regulation (EU) 2015/2283.

Dominique Turck, J. Castenmiller, S. de Henauw, K. I. Hirsch-Ernst, J. Kearney, A. Maciuk, I. Mangelsdorf, H. J. Mcardle, A. Naska, C. Pelaez, et al.

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Safety of Cetylated Fatty Acids as a Novel Food pursuant to Regulation (EU) 2015/2283

EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA), Dominique Turck, Jacqueline Castenmiller, Stefaan De Henauw, Karen Ildico Hirsch-Ernst, John Kearney, Alexandre Maciuk, Inge Mangelsdorf, Harry J McArdle, Androniki Naska, Carmen Pelaez, Kristina Pentieva, Alfonso Siani, Frank Thies, Sophia Tsabouri, Marco Vinceti, Francesco Cubadda, Karl Heinz Engel, Thomas Frenzel, Marina Heinonen, Rosangela Marchelli, Monika Neuhäuser-Berthold, Morten Poulsen, Yolanda Sanz, Josef Rudolf Schlatter, Henk van Loveren, Wolfgang Gelbmann and Helle Katrine Knutsen

Abstract

Following a request from the European Commission, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver an opinion on an application for cetylated fatty acids as a novel food (NF) pursuant to Regulation (EU) 2015/2283. The NF concerns primarily a mixture of cetylated myristic acid and cetylated oleic acid synthesised from cetyl alcohol, myristic acid and oleic acid, and to a lesser degree, other cetylated fatty acids and other compounds from olive oil. The NF is intended for use in food supplements for the general adult population. The highest dose tested in a subchronic toxicity study in rats, i.e. 4,500 mg/kg per day, was considered to be the no-observed-adverse effect level. By applying the default uncertainty factor of 200 as suggested by the EFSA Scientific Committee (2012), and considering a default body weight of 70 kg for the adult target population, this would result in an intake of 1.6 g per day, which is lower than the maximum intake proposed by the applicant (i.e. 2.1 g per day). The Panel concludes that the NF, cetylated fatty acids, is safe at an intake of 1.6 g per day for the intended target population, i.e. adults.

Keywords: Novel Foods, cetylated fatty acids, palmitoyl myristate, palmitoyl oleate, cetyl alcohol, hexadecane-1-ol, food supplements

Requestor: European Commission

Question number: EFSA-Q-2020-00422

Correspondence: nda@efsa.europa.eu

Panel members: Dominique Turck, Jacqueline Castenmiller, Stefaan De Henauw, Karen Ildico Hirsch-Ernst, John Kearney, Helle Katrine Knutsen, Alexandre Maciuk, Inge Mangelsdorf, Harry J McArdle, Androniki Naska, Carmen Pelaez, Kristina Pentieva, Alfonso Siani, Frank Thies, Sophia Tsabouri and Marco Vinceti.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

On 4 June 2020, the company Pharmanutra S.p.A. submitted a request to the European Commission in accordance with Article 10 of Regulation (EU) 2015/22831 to authorise placing on the Union market of cetylated fatty acids (CFA) as a novel food.

The application requests to authorise use of cetylated fatty acids in food supplements as defined in Directive 2002/46/EC2, excluding food supplements for infants and young children.

The applicant has also requested data protection in accordance with Article 26 of Regulation (EU) 2015/2283.

In accordance with Article 10(3) of Regulation (EU) 2015/2283, the European Commission asks the European Food Safety Authority to provide a scientific opinion on CFA as a novel food.

In addition, the European Food Safety Authority is requested to include in its scientific opinion a statement as to if, and if so to what extent, the proprietary data for which the applicant is requesting data protection was used in elaborating the opinion in line with the requirements of Article 26(2)(c) of Regulation (EU) 2015/2283.

1.2. Additional information

In 2010, EFSA published an opinion of the NDA Panel on the safety of 'Cetyl Myristoleate Complex (CMC)' (EFSA NDA Panel, 2010). Besides excipients (corn starch and silicon dioxide) that Novel Food was composed of an oil which contained mainly cetylated fatty acids (CFAs). Cetylated fatty acids are fatty acids esterified with the long-chain (C16) aliphatic (linear), saturated, primary alcohol cetyl alcohol (1-hexadecanol, palmityl alcohol). The oil's major components were the esters cetyl (=palmityl) myristate and cetyl (palmityl) myristoleate (each about 40% of that oil). Considering the absence of appropriate data on absorption, distribution, metabolism and excretion, particular for CFAs, which potentially may not be hydrolysed in the human gut and be absorbed intact as an ester, and no subchronic toxicity study reported, the Panel considered that the toxicological data provided were insufficient. Regarding genotoxicity, the EFSA NDA Panel noted references from the literature provided by the applicant which reported negative results for myristic acid and cetyl alcohol.

In 2013, in their response to a request from the Commission to review and update its opinion from 2010, additional references and a study report of a new subchronic 90-day oral toxicity with 'CMC' study were taken into account by the EFSA NDA Panel, 2013. The no observed adverse effect levels (NOAELs) of long-chain alcohols, including cetyl alcohol in repeated toxicity studies (OECD, 2006; Veenstra et al., 2009) were noted to be all in a similar range, with the lowest value at 750 mg/kg body weight (bw) per day based on cetyl alcohol. The NDA Panel noted that apart from information on the safety of the hydrolysis-derived moieties (i.e. cetyl alcohol and the concerned fatty acids), and in the absence of sufficient data on kinetics of hydrolysis, adequate safety information on the parent compound 'CMC' (i.e. CFAs) remained relevant. The NDA Panel also noted the shortcomings of the provided subchronic toxicity study and reiterated their conclusion from 2010 that the safety of 'CMC' has not been established (EFSA NDA Panel, 2013).

In 2009 and 2012, the EFSA CONTAM Panel assessed also the toxicological profile of fatty alcohols including cetyl alcohol, and esters made of fatty acids (including myristic acid, myristoleic acid, oleic acid and others) and fatty alcohols (including cetyl alcohol) (EFSA CONTAM Panel, 2009, 2012). However, these assessments were conducted in another context, i.e. regarding residual substances in previous cargoes for edible fats and oils with very low potential exposure to these substances.

2. Data and methodologies

2.1. Data

The safety assessment of this NF is based on data supplied in the application and information submitted by the applicant following EFSA request for supplementary information.

Administrative and scientific requirements for NF applications referred to in Article 10 of Regulation (EU) 2015/2283 are listed in the Commission Implementing Regulation (EU) 2017/2469[1].

A common and structured format on the presentation of NF applications is described in the EFSA guidance on the preparation and presentation of an NF application (EFSA NDA Panel, 2016). As

[1] Commission Implementing Regulation (EU) 2017/2469 of 20 December 2017 laying down administrative and scientific requirements for applications referred to in Article 10 of Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. OJ L 351, 30.12.2017, pp. 64–71.

indicated in this guidance, it is the duty of the applicant to provide all of the available (proprietary, confidential and published) scientific data (including both data in favour and not in favour) that are pertinent to the safety of the NF.

This NF application includes a request for protection of proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283. The data requested by the applicant to be protected comprise unpublished study reports on: an in vitro bacterial reverse mutation assay (Thompson, 2017), an in vitro micronucleus test (Morris, 2017), a subacute (Piras, 2019) and a subchronic toxicity study (Piras, 2020) including a summary table of statistically significant observations in toxicity studies (Appendix B3). Data protection was also requested for Annex III (certificates of analysis, batch testing and methods of analysis) and annex IV (stability data).

2.2. Methodologies

The assessment follows the methodology set out in the EFSA guidance on NF applications (EFSA NDA Panel, 2016) and the principles described in the relevant existing guidance documents from the EFSA Scientific Committee. The legal provisions for the assessment are laid down in Article 11 of Regulation (EU) 2015/2283 and in Article 7 of the Commission Implementing Regulation (EU) 2017/2469.

This assessment concerns only the risks 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.

3. Assessment

3.1. Introduction

In accordance to Article 3 of the Novel Food Regulation (EU) 2015/2283, the NF falls under category (i), i.e. food with a new or intentionally modified molecular structure, where that structure was not used as, or in, a food within the Union before 15 May 1997.

3.2. Identity of the NF

The NF is a mixture composed of cetylated fatty acids (about 83%) which are esters made from cetyl alcohol (synonyms: 1-hexadecanol, palmityl alcohol) (CAS number: 36653-82-4) and fatty acids, primarily myristic acid (CAS number: 544-63-8) and oleic acid (CAS number: 112-80-1). It contains also smaller amounts of other cetylated fatty acids from esterification of other fatty acids (such as linoleic and palmitic acid) contained in refined olive oil (CAS number: 8001-25-0) and about 17% triglycerides.

3.3. Production process

The applicant provided the certificates of analysis (CoA) of the raw materials used. According to these files, all raw materials are food grade. The raw material 'myristic acid' contains 99.8% myristic acid ($\geq$ 99%) and maximum 1% shorter or longer FAs. 'Oleic acid' contains at least 78% oleic acid, maximum 13% linoleic acid (C18:2), maximum 6% palmitic acid (C16) and smaller amounts of shorter ($\leq$ C14) or longer ($\geq$ C20) FAs including traces of polyunsaturated FAs (e.g. $\leq$ 0.5% α-linolenic acid, C18:3). The CoA for cetyl alcohol indicated a minimum content of 98% and a maximum content of hydrocarbons of 0.5%.

According to the applicant, CFAs are manufactured in compliance with the principles of Hazard Analysis Critical Control Points (HACCP) and Good Manufacturing Practice (GMP) and are accredited with International Organization for Standardization (ISO) 9001:2015, 22000:2005, 14001:2015 and 18001:2007. The manufacturing process does not use any extraction solvents and includes heating and filtration steps to remove any potential contaminants. Furthermore, all starting materials used in the production of cetylated fatty acids are food grade.

Cetyl esters are first synthesised by reacting cetyl alcohol with myristic acid and oleic acid in the presence of a zinc catalyst under high temperature. After cooling, the refined cetyl esters undergo decolourisation, and are again mixed with olive oil at high temperature. Further deodorisation, neutralisation and filtration yield the final ingredient. The NF is stored in aluminium boxes.

The Panel considers that the production process is sufficiently described.

3.4. Compositional data

Five independently produced batches show that the NF consists mainly of the cetylated fatty acids cetyl myristate and cetyl oleate, and triglycerides (Table 1). The triglycerides and the oleic acid moiety

of cetyl oleate originate from olive oil. CoAs are provided. The analyses of the esters and the triglycerides were performed using gas chromatography with flame ionisation detection (GC-FID).

Table 1: Batch to batch analysis of the NF

Parameter	Method of Analysis	Batch Number				
		No 1	No 2	No 3	No 4	No 5
Colour (AHPA colour)	AOCS Ea9-65	< 600	< 600	< 600	< 600	< 600
Acid value (mg KOH/g)	AOCS Cd3d-63	0.8	0.7	0.7	0.4	0.8
Iodine value (g I2/100 g)	AOCS Cd1-25	30.3	30.5	32.2	30.1	30.1
Saponification value (mg KOH/g)	AOCS Cd3-25	134.4	138.2	138.6	141.3	136.6
Hydroxyl value (mg KOH/g)	AOCS Cd13-60	6.3	7.1	6.0	4.1	7.4
Ester content (%)	GC-FID	75.14	74.38	74.23	72.19	73.49
Cetyl oleate (%)	GC-FID	23.95	23.80	23.77	23.10	23.48
Cetyl myristate (%)	GC-FID	49.37	48.82	48.69	47.39	48.25
Triglycerides	GC-FID	22.83	23.81	24.82	24.85	22.84
Aluminium (mg/kg)	ICP-MS	1.57	1.26	1.03	1.49	1.70

APHA: American Public Health Association; AOCS: American Oil Chemists' Society; GC-FID: gas chromatography with flame ionisation detection; ICP/MS: inductively-coupled plasma mass spectroscopy; KOH: potassium hydroxide.

Following a request from EFSA about the source of aluminium, the applicant responded that the NF is stored in aluminium boxes.

The applicant provided also the results from one batch on the total fatty acid profile of the NF (Table 2).

Table 2: The total fatty acid composition of the NF

Fatty acid	Results
Oleic acid (%)	45.98
Myristic acid (%)	40.96
Linoleic acid (%)	7.97
Palmitic acid (%)	3.24
Stearic acid (%)	0.80
Palmitoleic acid (%)	0.44
Eicosenoic acid (%)	0.30
Lauric acid (%)	0.21
Eicosanoic acid (%)	0.11

The applicant provided also information on heavy metals from two independent batches (Table 3), showing low concentrations. In addition, five batches were also analysed for 3-monochloropropanediol (3-MCPD) and glycidyl fatty acid esters, indicating much lower concentrations than those maximally permitted in fats and oils according to Commission Regulation (EC) No 1881/2006 (Table 4).

Table 3: Heavy metals in two independent batches

Parameter	Batch number		Method of analysis
	No 6	No 7	
Arsenic (mg/kg)	< 0.005	NT	ICP-MS
Cadmium (mg/kg)	< 0.005	0.008	
Mercury (mg/kg)	< 0.005	< 0.005	
Lead (mg/kg)	0.008	< 0.005	
Nickel (mg/kg)	0.020	NT	

ICP-MS: inductively coupled plasma mass spectrometry; NT: not tested.

Table 4: Results for 3-MCPD and glycidyl fatty acid esters of five independent batches of the NF

Parameter	Batch number				
	No 8	No 2	No 3	No 4	No 5
3-MCPD (μg/kg)	< 10	n.d.	n.d.	n.d.	n.d.
Sum of 3-MCPD and 3-MCPD fatty acid esters, expressed as 3-MCPD (μg/kg)	n.d.	210	230	260	240
Glycidyl fatty acid esters expressed as glycidol (μg/kg)	340	300	400	320	290

n.d.: not detected; 3-MPCD: 3-monochloropropanediol.

The applicant also analysed, although for only one batch, dioxins (0.13 pg/g) and polychlorinated biphenyls, polycyclic aromatic hydrocarbons (1.3 μg/kg) and erucic acid (< 0.5 g/kg), showing concentrations which are not of concern.

Microbiological contamination (Table 5) was provided from five batches.

Table 5: Microbiological analyses of five independent batches of the NF

Parameter	Method of analysis	Specification	Batch number				
			No 1	No 2	No 3	No 4	No 5
Total aerobic microbial count (CFU/g)	ISO 4833	< 1,000	< 1,000	< 100	< 1,000	1,000	1,000
Escherichia coli (negative/g)	ISO 16649-2	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
Salmonella (negative/25 g)	ISO 21528-1/2	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
Staphylococcus aureus (negative/g)	ISO 6888-1	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
Yeasts and Moulds (CFU/g)	ISO 21527-1/2	< 100	< 100	< 10	< 100	< 100	< 100
Enterobacter (CFU/g)	ISO 6579	< 100	< 100	< 100	< 100	< 100	< 100

CFU: colony forming units; ISO: International Organization for Standardization.

Information was also provided on the accreditation of the laboratories that conducted the analyses presented in the application.

The Panel considers that the information provided on the composition of the NF is sufficient and does not raise safety concerns.

3.4.1. Stability

The applicant performed stability tests with five independently produced batches (1090l19/01–1090l19/05) of the NF. The tests were carried out at 25 ± 2°C for 12 months and under accelerated conditions (40 ± 2°C) for 9 months. The batches were analysed for the saponification and hydroxyl values and all microbiological and physico-chemical specification parameters indicated in Table 1 except for triglycerides. At the end of 12 and 9 months, respectively, all tested parameters were within the specification limits. Small changes of the saponification and hydroxyl value were noted, but are not of concern. The provided stability data indicate that the NF is sufficiently stable for at least 12 months under 25 ± 2°C.

The Panel considers that the data provided sufficient information with respect to the stability of the NF for a duration of 12 months.

3.5. Specifications

The specifications of the NF as proposed by the applicant are indicated in Table 6. The specification parameters are assessed using internationally recognised methods or are otherwise determined using internally developed and validated methods.

Table 6: Specifications of the NF

Parameter	Specification
Physical status at 25°C	Solid
Colour (APHA colour)	≤ 600
Acid value (mg KOH/g)	≤ 5
Iodine value (g I2/100 g)	30–50
Saponification value (mg KOH/g)	130–150
Hydroxyl value (mg KOH/g)	≤ 20
Ester content (%)	70–80
Cetyl oleate (%)	22–30
Cetyl myristate (%)	41–56
Microbiological criteria	
Total aerobic microbial count (CFU/g)	≤ 1,000
Yeasts and moulds (CFU/g)	≤100

APHA: American Public Health Association; CFU: colony forming units; KOH: potassium hydroxide.

Although the applicant has proposed to add aluminium as a parameter to the specification, when considering the source of aluminium, the low levels detected in the five batches (1.03–1.57 mg/kg NF) and the proposed use levels, the Panel considers that no specification maximum is needed for this parameter.

The Panel considers that the information provided on the specifications of the NF is sufficient and does not raise safety concerns.

3.6. History of use of the NF and/or of its source

3.6.1. History of use of the source

The applicant refers to the history of fatty acids in the European diet. Myristic acid is found in coconut oil, nutmeg butter, palm seed oil and milk fat, with small amounts detected in most animal and vegetable oils (CIR, 1987). The applicant also notes esterified oleic acids found in many vegetable oils and animal fats.

3.6.2. History of use of the NF

According to the applicant, the NF has no history of use outside of the EU, but a similar ingredient 'cetyl myristoleate complex (CMC)' has been authorised by the Korean Food and Drug Administration in 2009.

In its opinion from 2010 on another NF application for cetylated fatty acids, the Panel noted that according to the applicant of that application, the concerned NF was produced and sold in quantities over 10.000 kg CMC in the US between 1996 and 2008 with no adverse effects reported (EFSA NDA Panel, 2010).

In the US, synthetic fatty alcohols, including cetyl alcohol is a food additive permitted for direct addition to food for human consumption under certain conditions regarding production process, purity (at least 98%) and use (FDA, 2020).

In 1999, JECFA estimated the daily per capita intake of cetyl alcohol as a flavouring agent to be 3.6 μg/day (JECFA, 1999).

3.7. Proposed uses and use levels and anticipated intake

3.7.1. Target population

Following a request from EFSA to provide clarification regarding intended target population, the applicant clarified and confirmed that the target population is the general adult population only.

3.7.2. Proposed uses and use levels

The applicant proposes the use of the NF for food supplements at a use level of 2.1 g/day.

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

No ADME studies were provided for the NF or with other cetylated fatty acids. The applicant referred to the ADME on triglycerides, fatty acids and the EFSA Opinions on another, similar NF application ('CMC') (EFSA NDA Panel, 2010, 2013).

In 2008, the EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food (AFC) concluded on 40 different linear- and branched-chain aliphatic saturated primary esters with an alkyl chain length of up to C7, that these esters would undergo hydrolysis to yield their corresponding linear- or branched-chain aliphatic alcohols and linear carboxyl acids and that any remaining non-hydrolysed esters are expected to be absorbed rapidly from the gastrointestinal tract as well, after which they are also expected to be hydrolysed (EFSA AFC Panel, 2008).

In their Opinion from 2008, the EFSA AFC Panel also summarised available data on the fate of cetyl alcohol (*citation*): 'Data for the C16 aliphatic alcohol, hexadecan-1-ol (cetyl alcohol), indicate that of the 34% total absorbed (recovered in thoracic duct lymph, carcass, liver, expired CO_2, and urine) of a dose in corn oil administered by oral gavage to male Sprague-Dawley rats, 75% was found in the thoracic duct lymph after 24 h and that 85% of this material had been converted to fatty acid, presumed to be palmitic acid (Baxter et al., 1967). Another study corroborates this result in that cetyl alcohol was well absorbed (63–96%, based on the difference between amount administrated and amount recovered from the intestinal tract and faeces) when fed to rats. From 31% to 64% of the absorbed material was recovered from the thoracic lymph lipids. About 15% of this amount was present as unchanged cetyl alcohol. The remainder had been oxidised to palmitic acid and subsequently incorporated into triglycerides and phospholipids. The main part of this oxidation process took place during the passage of the lipids through the intestinal mucosa cells (Blomstrand & Rumpf, 1954).' According to the JECFA (1999), such linear alcohols (including cetyl alcohol) are rapidly absorbed from the gastrointestinal tract. Information on distribution and excretion of these compounds has not been submitted, but these flavouring substances can be expected to be extensively metabolised to substances that are easily excreted by animals and humans. According to JECFA (1999), cetyl alcohol is oxidised to hexadecanal which is rapidly oxidised to hexadecanoic acid which is metabolised via the fatty acid and tricarboxylic acid pathways.

In the opinion on the NF application 'CMC' from 2010, the NDA Panel noted that there is some information that long-chain fatty acid esters are hydrolysed but at a slow rate.

3.9. Nutritional information

Taking into account the composition of the NF and the proposed maximum intake of NF, i.e. 2.1 g per day, the Panel considers that the NF is not nutritionally disadvantageous.

3.10. Toxicological information

The applicant provided four toxicological studies performed with the NF, an *in vitro* bacterial reverse mutation test (Thompson, 2017) in compliance with the OECD Guidance No. 471 (OECD, 1997), an *in vitro* mammalian cell micronucleus test (Morris, 2017) in accordance with OECD Guidance No 487 (OECD, 2016), a non-GLP compliant 14 day subacute study (Piras, 2019) and a subchronic toxicity study following OECD Guidance No. 408 (OECD, 2018) (Piras, 2020). The respective study reports are unpublished and claimed proprietary by the applicant. The two *in vitro* genotoxicity studies and the subchronic toxicity study have been published in an article by Brilli and Tarantino (2020).

3.10.1. Genotoxicity

In the *in vitro* bacterial reverse mutation assay, *Salmonella typhimurium* strains TA1535, TA1537, TA98 and TA100 and *Escherichia coli* strain WP2uvrA were treated with the NF (Thompson, 2017). In this study, the test item was non-mutagenic at concentrations up to 5,000 μg/plate, in the absence or presence of metabolic activation. In an *in vitro* mammalian cell micronucleus test with human lymphocytes compliant with OECD Guidance No 487 and GLP with and without metabolic activation (Morris, 2017), the test item was not clastogenic or aneugenic.

Regarding cetyl alcohol, in 2010, the EFSA NDA Panel noted literature which showed negative results in genotoxicity studies (EFSA NDA Panel, 2010). In their assessment of long-chain alcohols, which covered also cetyl alcohol, OECD (2006) concluded that the category of long chain alcohols does not have genotoxic potential.

Taking into account the information provided on the raw material used, the production process, the composition of the NF and the test results of the two provided in vitro genotoxicity tests, the Panel considers that there are no concerns regarding genotoxicity.

3.10.2. Subacute toxicity

In a subacute toxicity study in rats, not in compliance with GLP and the respective OECD Guidance document, the NF was given by gavage at doses of 0, 1,600, 1,900, 2,200, 2,600 and 4,500 mg/kg per day for 14 days (Piras, 2019). A lower glucose plasma level in the high-dose group animals was the only potentially relevant finding.

3.10.3. Subchronic toxicity

The applicant provided a 90-day repeated dose toxicity study by Piras (2020) with Sprague-Dawley rats in compliance with the OECD principles of GLP (OECD, 1998) and according to OECD Guidance No 408 (OECD, 2018). Groups of 10 male and 10 female Sprague-Dawley rats received 0 (vehicle control = corn oil), 1,500, 3,000 or 4,500 mg/kg bw per day of the NF (batch number 1090L18/01) by oral gavage at a dose volume of 10 mL/kg bw per day, each as two separate daily doses (60–150 min apart) for 90 days. An additional 'reference control' group (comprising the same number of animals) received 4,500 mg/kg bw per day of a mixture of 24% myristic acid, 13% oleic acid derived from olive oil with a purity of 78%, and 63% refined olive oil. All formulations were heated to 51°C before dosing (dosing temperature of ~ 47°C), due to nature of the test article and reference control, which both otherwise solidified at lower temperatures. Additionally, five males and five females, in each of the vehicle control, high-dose and reference control groups were employed as recovery groups.

There were no deaths during the 90 days, no test item-related clinical signs observed in the weekly assessment and no ophthalmological changes observed at the end of the treatment. In the functional observation battery (FOB) assessments (including assessment of sensory reactivity to different stimuli, grip strength and motor activity) during the 11th week of the treatment, female rats of the mid- and high-dose group showed a statistically significant decrease in crossing motor activity. Since no such differences were observed for male animals, and no differences in the other endpoints of the FOB or other clinical sign in either sex, the Panel considered that the difference in the motor activity observed in the female rats of the mid and high dose was toxicologically not relevant.

There were no differences in body weight or food consumption between test item groups and the vehicle controls in both sexes. The fact that 4,500 mg of the vehicle control (corn oil) per kg bw per day represents a significant proportion of the daily energy intake of this group and that no difference in food consumption and body weight was observed among all groups, suggest that the cetylated fatty acids have been utilised as a source of energy.

Regarding clinical haematology and clinical chemistry assessments, the following parameters showed statistically significant differences between vehicle controls and test item groups: reduced (by about 3%) mean corpuscular haemoglobin concentration of high-dose males and the reference control males, and in females of the high-dose group (reduction by about 2%). Given the small differences and considering that there was no other affected endpoint related to red blood cells and haemoglobin, the Panel considers that these differences are toxicologically not relevant.

Compared to the vehicle control, there were further statistically significant differences such as: an increase of the prothrombin time in low-dose males and females, mid-dose males and reference control males and females, increased platelets in mid-dose females, increased glucose in low-dose females, reduced total protein and albumin in low- and mid-dose males and an increase of urea in low- and mid-dose males. In the absence of a dose–response relationship, and given that these findings were only seen in low- and mid-dose animals, the Panel considers these findings as incidental.

Statistically significant differences were observed between the vehicle control and the high-dose female rats: a lower urea (–26%), an increase in sodium (+6.1%) and a 43% higher plasma potassium concentration. The Panel notes the high variation for urea in plasma. It was statistically significant higher in the low- and mid-dose male rats relative to the vehicle control, but not in the high-dose male rats. Among the recovery groups, the potassium values were statistically significantly higher in both the female and male of the high dose and the reference control groups compared to the vehicle control group. While a 6.1% increase of sodium in the female high-dose group compared to the vehicle control was the only statistically significant difference among the measured sodium plasma concentrations in the three male and female treatment groups, statistically significant higher sodium was also reported for the recovery reference control groups in both sexes. Noting also that there were

no statistically significant differences in the absolute and relative kidney weights and in the urinalysis, and no histopathological findings in the kidney, the Panel considers that the reported statistical differences in the high-dose female rats for urea, sodium and potassium are not of toxicological relevance.

There were no statistically significant differences in thyroid hormones between controls and test item groups. There were no test item-related differences in absolute and relative organ weights, except for statistically significant increases for absolute and relative pancreas weights for females in all dose groups and a reduced urinary bladder weight for low-dose females. However, these differences were not associated with a dose response and no test item-related macroscopic and histopathological findings were observed in the pancreas.

Histopathological findings of potential relevance were observed in the salivary glands, stomach, aorta and lungs. In all groups, including the vehicle and reference control group, animals were found at the end of 13 weeks with acinar cell degeneration and/or atrophy of the salivary glands. Such effects in the salivary glands were also reported after the recovery period for animals of the vehicle and reference control, but not for the high-dose animals. Also in all groups, including recovery groups, animals were found with superficial disruption in the glandular part of the gastric mucosa. The incidence of this finding in the stomach was lowest in the vehicle control (11/20) and highest in the high dose (19/20) and reference control group (19/20). The number of affected animals was similar among the recovery groups (9/10 for the vehicle control; and 7/10 for both the high dose and the reference control). The Panel considers that these treatment-related effects were due to the gavage administration of the heated test materials.

The study report also shows that emphysematous pictures were seen in the lungs of animals in all groups with the lowest incidence in the vehicle control (1/20) and the highest incidence in the reference control group (11/20). There was no dose-response among the three dose groups: each 10 affected animals in the low and mid-dose, and 9 in the high dose. The incidence among the recovery groups was similar (each 7/10 in the vehicle and high-dose group and 8/10 in the reference control group). Lung emphysema has been reported as a common effect in the review by Iwarsson and Rehbinder (1993) when CO_2 is used as euthanasia methods in rats, mice and guinea pigs. The animals in the 90-day study were killed by CO_2. In the absence of a dose-response and noting the high incidence of this effect for the vehicle control recovery group, the Panel considers that the effects were not related to toxicity of any of the tested materials.

Regarding aorta, no animal in the vehicle control group, but 2/20, 1/20, 4/20 and 3/20 animals in the low, mid, high dose and the reference control group, respectively, had areas of early degeneration. Considering that 1) there was no dose-response, 2) that also three animals of the reference control and 3) that 1/10 animals of the vehicle and 1/10 animals of the reference control, but no animal of the high-dose group had such a finding after the recovery period, the Panel considers that these findings are not of toxicological relevance for the NF.

Overall, the Panel notes that histopathological findings in the three dose groups were seen in either one or both of the control groups at the end of 13 weeks and/or at the end of the recovery period. The Panel also notes that the findings in the high-dose groups and the reference control group were essentially the same. The Panel considers that the histopathological findings in the two control groups were not related to corn oil toxicity, the fatty acids and/or olive oil, but rather due to the treatment with the heated test materials administered by gavage and possibly also to the euthanasia with CO_2 (lungs) as performed in this study. The Panel is of the view that the same applies to the histopathological findings of the three test groups receiving the NF. This consideration is supported by the absence of any other statistically significant finding considered to be related to the administration of the test materials. Consequently, the Panel considers that the highest dose tested, i.e. 4,500 mg/kg bw per day of the NF, is the NOAEL of this subchronic study.

3.10.4. Human data

The Panel notes that there are no human studies conducted with the NF available.

3.11. Allergenicity

Cetyl alcohol is a known contact allergen (Aakhus and Warshaw, 2011; Gaul, 1969; Komamura et al., 1997; van Ketel, 1984). The Panel considers that is unlikely to cause allergenic reactions under the intended uses.

4. Discussion

The NF concerns a mixture composed of cetylated fatty acids (about 83%) made from cetyl alcohol, and myristic acid and oleic acid. The NF contains also smaller amounts of other cetylated fatty acids (such as linoleic and palmitic acid) from esterification of fatty acids contained in refined olive oil. While there is long and substantial history of human consumption of olive oil, oleic acid and also myristic acid, no such data exist for cetyl alcohol or cetylated myristic and cetylated oleic acid. In order to address potential subchronic toxicity, the applicant provided a 90-day study with rats. In the absence of toxicity related to the NF observed in this study, the Panel considers that the NOAEL of this subchronic study was the highest dose tested, i.e. 4,500 mg/kg bw per day. By applying the default uncertainty factor of 200 as suggested by the EFSA Scientific Committee (2012), and considering a default body weight of 70 kg for the adult target population, this would result in an intake of 1.6 g per day, which is lower than the maximum intake proposed by the applicant (i.e. 2.1 g per day).

5. Conclusions

The Panel concludes that the NF, cetylated fatty acids, is safe at an intake of 1.6 g per day for the intended target population, i.e. adults.

The Panel could not have reached the conclusion on the safety of the NF under the proposed conditions of use without the data claimed as proprietary by the applicant (Morris, 2017; Thompson, 2017; Piras, 2020) as well as a summary table of statistically significant observations in toxicity studies (appendix B3), annex III (certificates of analysis, batch testing and methods of analysis) and annex IV (stability data).

6. Steps taken by EFSA

1) On 20/07/2020 EFSA received a letter from the European Commission with the request for a scientific opinion on the safety of cetylated fatty acids as a novel food. Ref. Ares (2020) 3814185.
2) On 20/07/2020, a valid application on cetylated fatty acids as a novel food (NF 020/1828), which was submitted by Pharmanutra S.p.A., was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2020/1828) and the scientific evaluation procedure was initiated.
3) On 06/10/2020 and 12/02/2021, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4) On 16/10/2020 and 23/04/2021, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
5) During its meeting on 26/05/2021, the NDA Panel, having evaluated the data, adopted a scientific opinion on cetylated fatty acids as a NF pursuant to Regulation (EU) 2015/2283.

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Abbreviations

ADME	absorption, distribution, metabolism and excretion
AFC	Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food
AOCS	American Oil Chemists' Society
APHA	American Public Health Association
Bw	body weight
CAS	Chemical Abstract Services
CFA	cetylated fatty acids
CFU	colony forming units
CIR	Cosmetic Ingredients Review
CMC	Cetyl Myristoleate Complex
CoA	Certificate of Analysis
CONTAM	Panel on Contaminants in the Food Chain
FA	fatty acid
FDA	Food and Drug Administration
FOB	functional observation battery
GC-FID	gas chromatography with flame ionisation detection
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis Critical Control Points
ICP-MS	Inductively-coupled plasma mass spectrometry
ISO	International Organization for Standardization
JECFA	Joint FAO/WHO Expert Committee on Food Additives
KOH	potassium hydroxide
MCPD	monochloropropanediol
NDA	Panel on Nutrition, Novel Foods and Food Allergens
NOAEL	no observed adverse effect level
NF	Novel Food
OECD	Organisation for Economic Co-operation and Development
US	United States