SCIENTIFIC OPINION

Scientific Opinion on dihydrocapsiate[1]

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a scientific opinion on the safety of a synthetic dihydrocapsiate (DHC) as a food ingredient in the context of Regulation (EC) No 258/97 taking into account the comments and objections of a scientific nature raised by Member States. Dihydrocapsiate belongs to a group known as capsinoids which have been shown to occur naturally in a number of chilli and sweet peppers. The applicant intends to market DHC to food manufacturers as an ingredient for incorporation into foods of various categories at concentration levels varying from 8 to 2050 mg per kg. Considering the proposed uses the mean intake of synthetic DHC was estimated to be around 12 – 13 mg/day (8.1 mg/day for pre-school children); the 97.5th percentile intakes of adults and the elderly were estimated to be around 34 mg/day (18.5 mg/day for pre-school children). Calculations based on body weights resulted in the highest intakes being for pre-school children (mean: 0.6 mg/kg bw/day; 97.5th percentile: 1.3 mg/kg bw/day). The applicant has provided a range of toxicological studies with DHC. The Panel concludes that it has no safety concerns regarding genotoxicity. Studies on developmental toxicity in rats and rabbits using commercial grade DHC did not show adverse effects on pregnant animals or on foetal growth and development. The no-observed adverse effect level (NOAEL) of three subchronic oral toxicity studies in rats was consistently at 300 mg DHC/kg bw/day. The Panel is of the opinion that the margin of safety (MOS) in relation to the NOAEL of 300 mg/kg bw/day is sufficient, including the highest estimated intake of 1.3 mg/kg bw/day for preschool children. The Panel concludes that the novel food ingredient, DHC, is safe under the proposed uses and use levels.

KEY WORDS

Dihydrocapsiate, capsinoids, novel food, ingredient

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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 a scientific opinion on the safety of 'dihydrocapsiate (DHC)' as a food ingredient in the context of Regulation (EC) No 258/97 taking into account the comments and objections of a scientific nature raised by Member States.

Dihydrocapsiate belongs to a group known as capsinoids. They were first identified in the fruits of a non-pungent cultivar of pepper (*Capsicum annuum* L.) and have been shown to occure naturally in a number of chilli and sweet peppers. In contrast to the analogous capsaicinoids (with capsaicin as major representative), the vanilloid and the fatty acid moieties in the capsinoids are bound via an ester rather than an amide bond. The applicant developed a procedure enabling the production of synthetic DHC which is based on a lipase-catalysed esterification of vanillyl alcohol and 8-methylnonanoic acid. Batch testing confirmed that the product complies with the given specifications. The applicant provided sufficient information regarding the specification, manufacture, composition and stability of DHC.

The applicant intends to market DHC to food manufacturers as an ingredient for incorporation into a range of foods such as baked goods, beverages, confectionery, cereals and desserts, and other foods including ready-to-eat frozen meals, soup, sweeteners and salad dressings. The applicants intends DHC use levels of about 8 - 18 mg per kg in beverages, 20 - 40 mg in cereals and desserts, and 11 mg per kg in ready-to-eat frozen meals and soups; higher concentration levels are intended for chocolates (75 mg/kg), baked goods (100 mg/kg), salad dressings (164 mg/kg) hard candies (270 mg/kg), whitener/creamer (383 mg/kg), sugar-free gum (1134 mg/kg), sweeteners (2050 mg/kg). Based on these proposed uses and use levels, the applicant conducted an intake estimation based on individual consumption data collected in the United Kingdom. The mean intake of DHC was around 12 - 13 mg/day for school children, adults and the elderly, and 8.1 mg/day for pre-school children, respectively; the 97.5th percentile intakes of adults and the elderly were around 34 mg/day and 18.5 mg/day for pre-school children, respectively. Calculations based on body weights resulted in the highest intakes being for pre-school children (mean: 0.6 mg/kg bw/day; 97.5th percentile: 1.3 mg/kg bw/day).

The available data on absorption, distribution, metabolism and excretion (ADME) suggest that ingested DHC is rapidly metabolised in the gut of rats and humans to form vanillyl alcohol, vanillic acid and 8-methylnonanoic acid. After absorption, the first two are converted into glucuronide and/or sulphate conjugates in the liver and eliminated predominantly by the kidneys into the urine. Total excretion in the urine, faeces and expired air after 72 hr was 98.0 %. The methyl-branched fatty acid 8-methylnonanoic acid is likely to be subject to a degradation via ß-oxidation. Terminal ω-oxidation might constitute an alternative pathway.

The applicant has provided a range of toxicological studies with DHC including in vitro and in vivo genotoxicity studies. Based on the results of these studies and in the absence of structural alerts for genotoxicity, the Panel concludes that it has no safety concerns regarding genotoxicity. Studies on developmental toxicity in rats and rabbits using commercial grade DHC did not show adverse effects on pregnant animals or on foetal growth and development up to the highest dose administered (1000 mg/kg bw/day). In a 13-week oral toxicity study in rats with laboratory scale DHC several changes were observed at the highest dose level (1000 mg/kg bw/day) which indicate an effect on the liver. Administration of commercial grade DHC to rats for 13 weeks at the same dose level induced in part the same specific changes indicating an effect on the liver. In addition, commercial grade DHC induced additional changes indicative of an effect on the kidneys. No explanation for the observed differences could be provided by the applicant. The Panel concludes that the intermediate dose of 300 mg DHC/kg bw/day is the no-observed adverse effect level (NOAEL) in these 13-week studies. In a 26-week oral toxicity study, commercial grade DHC induced, at the highest dose level (1000 mg/kg

bw/day), similar changes regarding liver- and kidney-specific parameters as those observed in the 13-week study with commercial grade DHC. Furthermore, slightly higher incidences of histopathological changes in the liver and kidneys compared with the controls were identified in the high-dose group. Not all effects induced by the test material were reversed at the end of a 4-week recovery period. The Panel concludes that 300 mg DHC/kg bw/day is the NOAEL in this study.

Taking into account the estimated intake levels resulting from the uses as proposed by the applicant, the Panel is of the opinion that the margin of safety (MOS) in relation to the NOAEL of 300 mg/kg bw/day in the 26-week rat toxicity study is sufficient, including the highest estimated intake of 1.3 mg/kg bw/day for preschool children.

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

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

On 31 August 2010, the company Toxico-Logical Consulting Ltd on behalf of the company Ajinomoto Co. Inc. submitted a request under Article 4 of the Novel Food Regulation (EC) No 258/97 to place on the market 'Dihydrocapsiate' (DHC) as a novel food ingredient.

On 10 March 2011, the competent authorities of the United Kingdom forwarded to the Commission their initial assessment report, which came to the conclusion that the DHC meets the criteria for acceptance as a novel food.

On 13 April 2011, the Commission forwarded the initial assessment report to the other Member States. Several of the Member States submitted comments or raised objections.

In consequence, a Community Decision is now required under Article 7, paragraph of Regulation (EC) No 258/97.

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

- There is a contradiction in the specification data provided by the applicant who indicated that the MNA content in the end product may vary between 2 to 7 % and that the DHC content was of > 94 %.
- Stability tests on the novel ingredient were carried out at temperature between 5°C and - 20°C, but not at higher temperatures and not in products to which the ingredient is intended to be added.
- The level of details for the exposure assessment is insufficient.
- Consumption of one intended DHC portion provides a 25 times higher intake than average intake from natural foods, in the worst case scenario it could be up to 280 times higher.
- Ambiguous results from genotoxicity testing: positive results in an AMES test (Salmonella tiphymurium, TA100 strain) and chromosome aberration test with CHL fibroblasts, both without a metabolising system (S9-mix) and “equivocal” results in the comet assay versus negative results in two in vivo tests in rats and mice. However, the later two were not carried out under GLP conditions.
- It is unlikely that DHC is able to accumulate in the body and target TRPV-1 (transient receptor potential vanilloid subfamily member 1) receptors other than those located along the digestive tract. However, TRPV-1 receptors are activated by different stimuli, including derivatives belonging to the family of vanilloids. It is not possible, based on the available data, to determine whether these receptors are likely to be stimulated by the DHC metabolites at the doses envisaged by the applicant. Moreover, no neurotoxicity study has been carried out.
- Lack of data on the capacity of human cells for beta-oxidising 8-methyl nonanoic acid (MNA). MNA is a methylated nonanoic short-chain fatty acid, branched and with an odd number of carbons in the carbon chain. This type of fatty acid is not synthesised by the human body. Their synthesis is of bacterial origin. They are therefore likely to be present only at trace levels in fermented products or in animal products from ruminants (metabolism by the rumen bacteria). It is unclear whether human cells are capable of beta-oxidising the MNA, in particular where a large quantity is to be metabolised.
- Inadequate design of human studies, i.e. short duration, low dose and small number of subjects.
- Absence of a study in children, in whom the doses likely to be ingested are twice as high per kg body weight as in adults.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002, the European Food Safety Authority is asked to carry out the additional assessment for 'Dihydrocapsiate' as a novel food ingredient in the context of Regulation (EC) N° 258/97.

EFSA is asked to carry out the additional assessment and to consider the elements of a scientific nature in the comments raised by the Member States.

ASSESSMENT

In accordance with Commission Recommendation 97/618/EC, the novel food ingredient 'Dihydrocapsiate (DHC)' is allocated to Class 1.2, i.e. foods or food ingredients that are 'pure chemicals or simple mixtures which are not obtained from plants, animals or microorganisms that have been genetically modified. The source of the NF has no history of food use in the Community'. Accordingly, information related to the structured schemes I, II, III, IX, XI, XII and XIII of Commission Recommendation 97/618/EC has been submitted. In the text, these structured schemes are listed 1 to 7. The assessment is based on data supplied in the original application, the initial assessment by the competent authority of The United Kingdom, the concerns and objections of the other Member States and the responses of the applicant. It is noted that the novel food ingredient is intended by the applicant to be added to foods to enhance energy expenditure and fat oxidation, and for its sensory effects. This assessment concerns only risk that might be associated with consumption, and is not an assessment of DHC with regard to any claimed benefit.

1. SPECIFICATION OF THE NOVEL FOOD (NF)

The structure of dihydrocapsiate (4-hydroxy-3-methoxybenzyl 8-methylnonanoate; $C_{18}H_{28}O_4$; CAS-no: 205687-03-2) is shown in Figure 1.

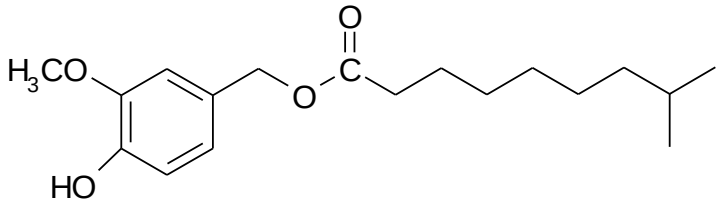

Figure 1: Structure of dihydrocapsiate

Dihydrocapsiate and the structurally-related capsiate (4-hydroxy-3-methoxybenzyl (*E*)-8-methyl-6-nonenoate) and nordihydrocapsiate (4-hydroxy-3-methoxybenzyl 7-methyloctanoate) belong to a group known as capsinoids. They were first identified in the fruits of a non-pungent cultivar of pepper (*Capsicum annuum* L.) (Yazawa et al., 1989; Kobota et al., 1998). Dihydrocapsiate and other capsinoids occur naturally in chilli and sweet peppers. In contrast to the analogous capsaicinoids, the vanilloid and the fatty acid moieties in the capsinoids are bound via an ester rather than an amide bond.

The applicant has developed a procedure enabling the production of synthetic DHC which is based on a lipase-cataylsed esterification of vanillyl alcohol and 8-methylnonanoic acid.

Specifications provided by the applicant are shown in Table 1. They comprise physico-chemical parameters of the novel food ingredient, a minimum assay value for DHC, and maximum contents of the employed starting materials. Taking into account the production procedure (see Section 2), the maximum contents of synthesis-related substances, of the solvent used for extraction and of heavy metal contaminants have also been specified.

According to the applicant, a residual amount of up to 6 % 8-methylnonanoic acid is retained in the final product; the resulting low pH prevents the dissociation of the phenolic moiety, and thus contributes to the stabilisation of the molecule.

The applicant provided compositional data for seven batches (between 10 and 20 kg each lot) of the novel food ingredient produced sequentially on pilot plant scale over a period of 3 months in 2006 (Table 2).

The analyses were conducted by two external accredited analytical laboratories which operated either in accordance with Good Laboratory Practice (GLP) and Good Manufacturing Practice (GMP), or in compliance with ISO/IEC 17025.

Table 1: Specifications for the novel food ingredient DHC as proposed by the applicant

Test item	Acceptance criteria	Method[a]
Description	Viscous, colourless to yellow liquid	JSFA VII
Identification (Infrared spectrum)	Absorption at wave numbers 2953, 2928, 2855, 1733, 1519, 1278, 1241, 1036, 818 and 798 cm-1	FCC V
Specific gravity	1.02 – 1.03	FCC V
Dihydrocapsiate (DHC)	≥ 94 %	HPLC-UV[b]
Starting materials		
8-Methylnonanoic acid	2 – 7 %	HPLC-UV[b]
Vanillyl alcohol	< 1 %	
Synthesis-related substances[c]	< 2 %	HPLC-UV[b]
Magnesium	< 1 ppm	JSFA VII
Copper	< 1 ppm	JSFA VII
Arsenic	< 1 ppm	JP XIV
Cadmium	< 1 ppm	FCC V
Lead	< 1 ppm	FCC V
Hexane	< 5 ppm	GC-FID[b]

[a] FCC V: Food Chemicals Codex Fifth Edition; JSFA VII: The Japan's Specifications and Standards for Food Additives Seventh Edition ; JP XIV: The Japanese Pharmacopoeia Fourteenth Edition
[b] analytical details of the methods have been provided
[c] vanillyl 6-bromohexanoate; (4-hydroxy-3-methoxybenzyl) 6-bromohexanoate
vanillyl decanoate; (4-hydroxy-3-methoxybenzyl) decanoate
vanillyl dihydrocapsiate; [4-(4-hydroxy-3-methoxybenzyloxy)-3-methoxybenzyl] 8-methylnonanoate
diacyl form; [4-(8-methylnonanoyl)-3-methoxybenzyl] 8-methylnonanoate

Table 2: Compositional data on seven lots of the novel food ingredient DHC produced at pilot plant scale in the period from June to August 2006

	Lot 060626	Lot 060705	Lot 060712	Lot 060720	Lot 060731	Lot 060807	Lot 060817
Dihydrocapsiate (%)	95.0	95.7	94.2	94.1	93.5	94.0	94.6
Starting materials							
8-Methylnonanoic acid (%)	2.0	2.0	3.3	3.4	5.8	4.3	4.8
Vanillyl alcohol (%)	0.04	0.03	0.03	0.03	< 0.025	< 0.025	< 0.025
Synthesis-related substances (%)	1.10	0.86	0.74	0.69	0.81	1.39	0.75
Vanillyl 6-bromohexanoate (%)	0.07	0.07	n.d.[a]	n.d.	n.d.	0.07	< 0.025
Vanillyl decanoate (%)	0.04	0.04	0.04	0.04	0.04	0.04	0.03
Vanillyl dihydrocapsiate (%)	0.60	0.50	0.48	0.47	0.48	0.73	0.37
Diacyl form (%)	0.14	0.12	0.11	0.09	0.17	0.42	0.24
Others (%)	0.25	0.13	0.11	0.09	0.12	0.13	0.085
Mass balance (% w/w)	98.14	98.59	98.27	98.22	100.11	99.69	100.15
Magnesium (mg/kg)	0.3	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2
Copper (mg/kg)	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2

[a] not detected

Arsenic (mg/kg)	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Cadmium (mg/kg)	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2
Lead (mg/kg)	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2
Hexane (mg/kg)	< 5	< 5	< 5	< 5	< 5	< 5	< 5
Specific gravity (g/ml)	1.030	1.030	1.028	1.028	1.024	1.026	1.025

The Panel considers the compositional data provided for the novel food ingredient as sufficient.

Data on stability

According to the applicant, the novel food ingredient is typically stored at -20°C in 1 kg amounts in aluminium pouches held in sealed containers. Data were provided on the stability upon storage at temperatures of -20°C, 5°C and 25°C up to 24 months (Table 3).

Table 3: Stability of the novel food ingredient (Lot NO: 070813) upon storage at different temperatures

	Months			
	0	**6**	**12**	**24**
	storage at -20°C ± 5°C			
Dihydrocapsiate (%)	95.7	95.7	94.6	95.1
Vanillyl alcohol (%)	0.12	0.12	0.11	0.11
8-Methylnonanoic acid (%)	3.9	3.9	3.9	4.0
Other synthesis-related substances (%)	0.59	0.59	0.57	0.66
	storage at 5°C ± 3°C			
Dihydrocapsiate (%)	95.4	95.2	94.7	94.3
Vanillyl alcohol (%)	0.11	0.34	0.37	0.58
8-Methylnonanoic acid (%)	3.9	4.1	4.2	4.5
Other synthesis-related substances (%)	0.71	0.62	0.63	0.70
	storage at 25°C ± 2°C[a]			
Dihydrocapsiate (%)	96.8	95.5	95.6	95.3
Vanillyl alcohol (%)	0.03	0.31	0.36	0.47
8-Methylnonanoic acid (%)	2.0	2.4	2.5	2.8
Other synthesis-related substances (%)	1.25	1.37	1.50	1.74

[a] relative humidity: 60 % ± 5 %

The applicant also checked the stability of DHC in a beverage subjected to a heating procedure of 80°C for 20 minutes. The test beverage contained high fructose corn syrup, citric acid, sodium citrate, sodium chloride, monopotassium phosphate, aspartame, acesulfam potassium, and liquid flavour (natural orange extract); the pH was 3.4. Dihydrocapsiate (0.5 %) was incorporated into the beverage via an emulsifying formulation. After the thermal treatment more than 95 % of the added DHC remained.

In response to Member States´ comments regarding the stability of the novel food ingredient in foods that will be heated prior to use, the applicant checked the stability of DHC upon heating for up to 30 minutes at temperatures of up to 200°C. As shown in Table 4, the novel food ingredient exhibited sufficient stability up to 150°C; treatments at 200°C resulted in significant degradation.
According to the applicant, DHC could be sprayed onto baked foods, for example biscuits, after the baking step in order to avoid temperatures of more than 150°C, and to maintain its stability and sensorial properties.

Table 4: Recovery of DHC upon heating of the novel food ingredient for up to 30 min. at 200°C

Heating temperature	**Heating time (min)**			
	0	10	20	30

		dihydrocapsiate (%)		
80°C	100	100.1	99.4	99.7
100°C	100	99.9	99.4	98.8
120°C	100	99.1	99.1	98.5
150°C	100	98.7	97.7	96.2
200°C	100	75.5	65.8	55.3

The Panel considers that the data provided sufficient information to demonstrate the stability of the novel food ingredient at a temperature of 25°C for up to 24 months. The Panel also noted that DHC is stable at temperatures of up to 150°C for 30 minutes.

2. EFFECT OF THE PRODUCTION PROCESS APPLIED TO THE NF

The synthesis of DHC is based on an enzyme-catalyzed esterification of vanillyl alcohol and 8-methylnonanoic acid.

The starting material, 8-methylnonanoic acid, is prepared from isobutyl bromide and 6-bromohexanoic acid ethyl ester through a Grignard coupling reaction and de-protection process. According to a certificate of analysis provided by a manufacturer, the purity (gas chromatography) of the material was ≥ 99.0 %. Vanillyl alcohol is obtained by reduction of vanillin. According to the certificate of analysis provided by a manufacturer, the mininum assay was 98.8 %.

8-Methylnonanoic acid is esterified with vanillyl alcohol (V-OH) using a lipase (Novozym® 435 FG, recently renamed Lipozyme 435; produced by Novozymes Denmark) as catalyst. A certificate including an approval reference of the Danish Veterinary and Food Administration was provided.

The enzyme is employed in immobilised form on an inert methacrylate carrier. According to studies performed by the enzyme producer, there is no release of protein or other materials from the carrier material under normal use. As further reassurance, the applicant implemented three post-reaction filtration steps in the production process, using filters of 5 µm porosity. The size of the Novozym® 435 FG granulate product is approximately 500 µm (range 150 – 190 µm); thus the filtration steps are considered to eliminate any granulate from the final product. Actual analytical data regarding the absence of protein residues have not been provided.

Following the esterification, n-hexane is employed to quench the reaction, as well as for the subsequent extraction step.

The reaction vessels are made of high grade stainless steel typical of pharmaceutical and food grade standard manufacturing plants. The scale-up to commercial scale is not considered to impact the quality of the resulting DHC or its ability to comply with the specification of the resulting commercial grade DHC food ingredient.

The Panel concludes that the production process is sufficiently described by the applicant and does not raise concerns.

3. HISTORY OF THE ORGANISM USED AS A SOURCE

Not applicable.

4. ANTICIPATED INTAKE/EXTENT OF USE OF THE NF

The applicant intends to market DHC to food manufacturers as an ingredient for incorporation into a range of foods such as baked goods, beverages, confectionery, cereals and desserts and other foods including ready-to-eat frozen meals, soup, sweeteners and salad dressings (Table 5). The proposed use levels have been calculated so that each portion of a given food will contain 3 mg of DHC. In the absence of a standard list of portion sizes, average quantities consumed per serving occasion by adults, according to data from the UK National Diet and Nutrition Survey (NDNS) (Office for National Statistics, 2005) have been used as the basis for the calculations (Table 5).

The Panel notes that the food categories of interest include those which could be consumed by pre-school children as well as school children, adults and the elderly.

Table 5: Intended uses and use levels of DHC

Food Category	Average portion size (g)*	Use level to provide 3 mg per serving (mg/kg)
Baked goods		
Cereal bars	33	90.1
Biscuits & cookies	33	90.5
Crackers	32	94.7
Rice-based snacks	25	119.0
Beverages		
Dilutable drinks	250	12.0
Carbonated drinks	267	11.2
Energy drinks	368	8.1
Fruit juice-based drinks	241	12.5
Drink mixes	209	14.3
Coffee-based drinks	212	14.1
Meal replacement drinks	320	9.4
Flavoured water - still	285	10.5
Tea-based drinks	208	14.4
Vegetable juice	169	17.8
Confectionery		
Sugar-free gum	3	1133.9
Hard candy	11	269.8
Chocolate confectionery	40	74.8
Cereals and desserts		
Frozen ices	86	34.8
Frozen dairy desserts	82	36.5
Pudding mixes	140	21.4
Yogurt (not fruit)	117	25.7
Yogurt (fruit-flavoured)	142	21.2

Instant oatmeal cereal	123	24.4
Other cereals	71	42.2
Other foods		
Ready-to eat frozen meals	276	10.9
Soup	280	10.7
Whitener/creamer	8	382.8
Replacement meal	265	11.3
Vegetable protein	61	49.5
Salad dressing	18	164.3
Sweeteners	1	2049.6

* Average quantity consumed per serving occasion, based on UK NDNS data for adults (Office for National Statistics, 2005).

Based on these proposed uses and use levels, the applicant conducted an intake estimation based on individual consumption data collected by UK NDNS surveys (Office for National Statistics, 2005; UKDA, 1995, 2001). The mean estimated intake of DHC was around 12 – 13 mg/day (8.1 mg/day for pre-school children); the 97.5th percentile intakes of adults and the elderly were around 34 mg/day (18.5 mg/day for pre-school children). Calculations based on body weights resulted in the highest intakes being for pre-school children (mean: 0.6 mg/kg bw/day; 97.5th percentile: 1.3 mg/kg bw/day).

Table 6: Estimated intakes* based on use levels providing 3 mg of DHC in an average portion for adults

	DHC intake, mg/day				DHC intake, mg/kg bw/day			
Age group	Mean	P90	P95	P97.5	Mean	P90	P95	P97.5
Adults	12.3	23.3	29.5	33.8	0.2	0.3	0.4	0.4
Pre-school children	8.1	13.1	15.7	18.5	0.6	0.9	1.1	1.3
School children	12.8	19.9	23.2	26.2	0.4	0.7	0.8	0.9
Elderly	11.7	23.2	29.2	34.2	0.2	0.3	0.4	0.5

* For consumers only

5. INFORMATION FROM PREVIOUS EXPOSURE TO THE NF OR ITS SOURCE

5.1. Natural occurrence in the human diet

Dihydrocapsiate is part of the human diet as it is naturally present in peppers. It has first been identified in the fruits of a non-pungent cultivar, CH-19 Sweet, of pepper (*Capsicum annuum* L.) (Yazawa et al., 1989; Kobata et al., 1998). Capsinoids have been shown to occur naturally in most chilli peppers (*Capsicum annuum*, *Capsicum frutescens*, *Capsicum chinense*, *Capsicum baccatum*, *Capsicum pubescens*) and sweet peppers like paprika (*Capsicum annuum* var. *annuum* L.). The contents of DHC and capsiate have been determined in various *Capsicum* species (Singh et al., 2009). According to the applicant, the average concentration of DHC in the analysed *Capsicum* samples (n = 77) was 19 mg/kg; the 90th, 95th and 97.5th percentiles were 43, 48 and 68 mg/kg, respectively.

Capsicum fruits and peppers have a long history of human consumption. Chillies are among the oldest cultivated crops of South and Central-America. The wild Capsicum annuum var. aviculare is harvested and sold in the marketplace alongside the larger-fruited domesticated chillies. Sweet peppers are widely consumed and cultivated in Hungary and Bulgaria and in the Mediterranean countries Spain, France and Italy. They are also widely grown and consumed in North Africa, California and New Mexico, as well as in the tropics and the Caribbean.

Based on FAO Food Balance Sheet data for capsicum from 2004 and 2005 for 157 countries, the applicant estimated that DHC intakes could range from 0 in countries where capsicums do not form part of the diet up to 5 to 16 mg/day (0.07 – 0.27 mg/kg bw/day) in some European countries such as Bosnia and Herzegovina and Hungary where capsicums are consumed in larger quantities. DHC intakes have been calculated for average consumers assuming average capsicum consumption and average levels of DHC (19 mg/kg).

For the United Kingdom, the applicant estimated based on NDNS consumption data of peppers for adults that the typical daily intake of DHC of natural occurance ranges from approximately 0.01 to 0.06 mg/kg bw/day.

Vanillyl alcohol is being used as a flavouring substance in the EU. It was evaluated by JECFA at its 57th meeting (JECFA, 2002). The EFSA Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) agreed with this evaluation and considered vanillyl alcohol not to be of safety concern at the estimated level of intake as a flavouring substance (EFSA, 2008).

5.2. Previous exposure of DHC via food supplements

Dihydrocapsiate has been determined to be generally regarded as safe (GRAS) at 1 and 3 mg per serving in the United States of America (USA), and these determinations were reviewed by the US Food and Drug Administration who provided "no objection" Agency response letters in 2009 and 2010. A chilli pepper extract, known as CH-19 Sweet extract, contains capsinoids of which DHC represents approximately 20 %. This extract is sold as a food supplement in the USA and Japan. The Panel notes that the applicant has not provided consumption data.

6. NUTRITIONAL INFORMATION ON THE NF

Dihydrocapsiate manufactured by the applicant is identical to the DHC found as a component of the capsinoids contained in Capsicums. The Panel considers that both synthetically produced DHC and DHC naturally occurring in the diet do not have a nutritionally relevant role in the human diet, and that the consumption of the novel food ingredient is not nutritionally disadvantageous.

7. MICROBIOLOGICAL INFORMATION ON THE NF

According to the information provided by the applicant, DHC is produced by chemical synthesis under GMP with good hygienic practices, and stored using tightly sealed heavy-duty bags made of Polyethylene Terephthalate/Aluminum foil/Oriented nylon/Liner Low Density polyethylene at -20ºC; the possibility of any significant microbiological contamination during either production or storage is considered remote.

For four of the batches of the novel food ingredient produced at pilot scale (Table 2), the total aerobic count was < 3000 Colony Forming Units (CFU)/g, and the counts for yeast/mould were < 100 CFU/g. No *E. coli* was detected in any of the four batches investigated. The test was conducted according to the method of Specifications and Standards for Foods, Food Additives, Food Additives General Tests

"Microbial Limit Test" (Notification No. 370, 1959), Ministry of Health and Welfare of Japan. The data provided do not raise concerns with regards to a microbial risk.

8. TOXICOLOGICAL INFORMATION ON THE NF

The applicant has carried out a number of in vitro and animal studies which have been also published in scientific literature. Some were carried out using synthetic DHC produced on a commercial scale by the applicant, some using laboratory-scale DHC. In addition, the applicant has provided studies with sweet pepper extract (CH-19 Sweet). Except for a pharmacokinetic study these examinations were not considered relevant for the safety assessment since the material tested cannot be regarded as representative for the product intended to be marketed (sweet pepper extract contains 7-9 % capsinoids of which only 20 % is DHC).

The toxicological data comprise pharmacokinetic studies, genotoxicity studies (tests for gene mutations in bacteria; tests for chromosomal aberrations in chinese hamster cells; micronucleus tests in mice, gene mutation tests using transgenic rats and comet assay in rats), a single dose oral toxicity study in mice, repeated dose oral toxicity tests with DHC administered to rats for 13 and 26 weeks, and studies on developmental toxicity in rats and rabbits.

8.1. Absorption, distribution, metabolism and excretion (ADME) data

The applicant provided a pharmacokinetic study using ^{14}C-DHC labelled at the vanillic alcohol moiety, which was carried out in male rats (Nemoto, 2005; Bernard et al., 2010). In this study, groups of two or three animals received the test material diluted in medium chain triglycerides (MCT) by gavage at a single dose of 10 mg/kg bw. Radioactivity was determined in plasma, urine, bile, faeces and expired air, carcass as well as specific organs and tissues. In addition, the metabolite profile in plasma was analysed by HPLC.

After a single oral administration of ^{14}C-DHC to rats, the radioactivity concentration in plasma reached a maximum (C_{max} 1870 ng eq./mL) at 0.67 hr (t_{max}) and then declined with an apparent half-life of 2.4 hr ($t_{1/2}$). The AUC (0-8 hr) and AUC (0-∞) were 6745 and 7581 ng eq. hr/mL, respectively. Total excretion in the urine, faeces and expired air was 94.1 % and 98.0 % up to 24 and 72 hr, respectively. Excretion was highest in the urine, i.e. 27.5 %, 57.9 %, 75.1 % and 78.2 % of the dose up to 4, 8, 24 and 72 hr after administration, respectively. Excretion in the faeces and expired air was 19.4 % and 0.5 % of the dose up to 72 hr, respectively. Residual radioactivity in the carcass after 72 hr was 4.0 % of the dose. When measured at 15 and 30 min after administration, radioactivity was highest in the kidneys, followed by the stomach and small intestine, the liver, plasma and blood. At 2 hr radioactivity was again highest in the kidneys followed by the caecum, stomach and small intestine, plasma, blood, liver and large intestine. At 6 hr the concentration of radioactivity in the tissues showed a marked decrease except for the stomach, large intestine and caecum. At 24 hr after administration the concentrations had decreased considerably in all organs. No residual radioactivity was detected in the brain.

In the HPLC analysis of plasma (samples from 15 and 30 min, 2 and 6 hr after administration) no DHC was detected but six metabolites designated RP1 – RP6 were identified. The main metabolites at all time points were RP2 (23.8 %, 28.3 %, 15.5 %, 21.4 % of the radioactivity) and RP3 (46.4 %, 49.4 %, 56.8 %, 20.8 %). The other metabolites were RP4 (detected at all time points; 2.4 - 3.8 %), RP1 (at 30 min, 2 and 6 hr; 1.8 – 4.0 %), RP5 and RP6 (detected at 6 hr; approximately 2.6 %). HPLC data obtained after treatment with ß-glucuronidase/arylsulfatase and the use of a ß-glucuronidase inhibitor suggested that RP2 is a glucuronide conjugate of vanillyl alcohol, RP3 is a sulphate conjugate of vanilyl alcohol and RP4 is a sulphate conjugate of vanillic acid. These data suggest that ingested DHC is metabolised in the gut to form vanillyl alcohol, vanillic acid and 8-methylnonanoic

acid. Vanillyl alcohol and vanillic acid are converted in the liver into glucuronide and sulphate conjugates, and eliminated predominantly by the kidneys into the urine.

The Panel considers that 8-methylnonanoic acid may be subject to degradation via ß-oxidation; however, there are no experimental data available on the human capacity to metabolise this methyl-branched fatty acid. Terminal ω-oxidation might constitute an alternative pathway, resulting in polar metabolites that can be excreted as conjugates (Wanders et al., 2010).

The applicant has also provided the results of pharmacokinetic studies in male rats using a sweet pepper extract (CH-19 Sweet) containing 7.5 % capsinoids (DHC, capsiate, nordihydrocapsiate), 20% of which are DHC (Shirai et al., 2010a, b). Sweet pepper extract was administered by gavage to fasted animals at a single dose of 10 or 100 mg/kg bw. Blood samples were obtained from the portal vein and aorta at 5, 15 and 30 minutes and 1, 2 and 4 hours after administration and analysed for capsinoids using Liquid Chromatography Tandem Mass Spectroscopy (LC-MS/MS) and for vanillyl alcohol using HPLC. The plasma levels of capsinoids were below the limit of quantification at all time points examined (LOQ 50 ng/ml for each of the capsinoids). Vanillyl alcohol (V-OH) – the metabolite common to the three capsinoids – was barely detectable in plasma (only at 5 minutes after administration). In portal vein plasma V-OH reached maximum concentrations 30 min after administration (C_{max} 0.16 and 1.48 µg/mL after administration of 10 and 100 mg extract/kg bw, respectively). Significant levels of sulfate and glucuronide conjugates of V-OH were detected in plasma from the aorta. These results further support the conclusions of the previous study that orally administered DHC is rapidly hydrolysed in the gastrointestinal tract. The metabolite V-OH is absorbed and converted in the liver to sulphate and glucuronide conjugates before entering the post-hepatic systemic blood.

In a human study, groups of 8 apparently healthy male subjects received a single oral dose of sweet pepper extract (CH-19 Sweet) containing 15 or 30 mg of capsinoids, corresponding to 4 and 8 mg DHC, respectively (Nakamura, 2006; Bernard et al., 2008a). The respective control groups (n = 4) received a placebo. Blood samples were taken at 15 and 30 minutes, 1, 2, 4, 8 and 24 hours after administration. The levels of capsinoids and V-OH in blood plasma were below the lower limit of quantification at all time points (LLOQ 10 and 50 ng/mL, respectively). These results suggest that also in humans capsinoids are rapidly hydrolysed in the gastrointestinal tract and further metabolised.

The potential effects of capsinoids, i.e. DHC, capsiate and nordihydrocapsiate, and of the capsaicinoid capsaicin, on the major human drug metabolising isozyme CYP3A4 were examined in vitro (Takahashi, 2005; Takanohashi et al., 2010). There was no inhibition of CYP3A4 by DHC and the other two capsinoids (<100 µmol/L) whereas capsaicin was clearly shown to inhibit this enzyme (50 % of the activity was lost at 21.5 µmol/L).

In summary, the available data suggest that ingested DHC is rapidly metabolised in the gut of rats and humans to form vanillyl alcohol, vanillic acid and 8-methylnonanoic acid. After absorption the first two are converted into glucuronide and/or sulphate conjugates in the liver and eliminated predominantly by the kidneys into the urine. Total excretion in the urine, faeces and expired air after 72 hr was 98.0 %. The methyl-branched fatty acid 8-methylnonanoic acid is likely to be subject to degradation via ß-oxidation. Terminal ω-oxidation might constitute an alternative pathway. The available animal and human data show that the potential risk of DHC accumulation in the fatty tissue or the brain can be considered negligible.

8.2. Genotoxicity studies

Genotoxicity test data have been provided (1) for laboratory scale DHC: bacterial mutation assay, in vitro chromosome aberration test, in vivo mouse micronucleus test, in vivo Comet assay in rats, (2) for commercial grade DHC: gene mutation assay in transgenic rats, in vivo mouse micronucleus test,

and (3) for CH-19 sweet extract: bacterial mutation assay, in vitro chromosome aberration test, in vivo mouse micronucleus test.

The CH-19 sweet extract contained low levels of capsinoids (7.5 % capsinoids of which 20 % is DHC); the results from these tests, albeit all negative, are not considered relevant for the safety assessment of DHC. The laboratory scale DHC and commercial grade DHC contained high levels of DHC: 95.8 % and 94.0 %, respectively, and are considered relevant.

8.2.1. Genotoxicity tests on laboratory scale DHC

A reverse mutation test in bacteria was carried out, in accordance with Japanese guidelines of the Industrial Safety and Health Law (Shimada, 2006; Bernard et al., 2008b). Four *Salmonella typhimurium* strains (TA98, TA100, TA1535 and TA1537) and one *Escherichia coli* strain (WP2uvrA) were used with and without metabolic activation (S9 mix). The results showed some evidence for an increase in revertant colonies in TA 100 cells treated in the absence of S9 mix at dose levels above 100 µg/plate. There was no evidence for an effect in the DHC-treated plates tested with S9 mix. In the definitive assays, up to 1000 and 2500 µg/plate were tested without and with S9 mix, respectively. In TA100 without S9 mix, where there was a dose-dependent two-fold or more increase in the number of revertant colonies as compared to control, no mutagenic activity was observed with or without metabolic activation for any of the other tested strains. The Panel considers that DHC was positive in strain TA 100 in the absence of S9 mix.

An in vitro chromosome aberration assay was carried out in accordance with the Japanese guidelines of the Industrial Safety and Health Law (Masumori, 2006; Bernard et al., 2008). Chinese hamster lung fibroblasts (CHL/IU cells) were tested with and without metabolic activation (S9 mix). The test substance was diluted in dimethylsulfoxide (DMSO). Doses up to 324 µg/ml were tested without S9 mix and continuous treatment. Up to 1500 µg/ml was tested for short term exposures with S9 mix. Doses above 194 µg/ml in the without S9 mix short and long term studies could not be used for metaphase analysis due to evident cytotoxicity and the lack of metaphase cells. Mitotic cells were scored at 3 dose levels as follows: 70.0, 117 and 194 µg/mL for the short-term treatment without S9 mix, 540, 900 and 1500 µg/ml for the short-term treatment with S9 mix, and 70.0, 117 and 194 µg/ml for the 24-hour continuous treatment without S9 mix. In the short-term treatment with S9 mix, the incidence of cells with chromosome aberrations (structural aberrations and numerical aberrations) was less than 5%, i.e. chromosome aberrations were not induced. In the short-term assay without S9 mix, an incidence of cells with chromosome aberrations (structural aberrations and numerical aberrations) was observed at 194 µg/ml with figures of 9.0 % and 8.0 %. In this treatment, dose dependent decreases in relative cell growth were seen, and the relative growth rate was only 38.6% at 194 µg/ml, which was the highest dose used in the evaluation due to frank cytotoxicity above. In the 24-hour continuous treatment without S9 mix, an increased incidence of cells with chromosome structural aberrations occurred at 194 µg/ml, where there was again a significant reduction in relative cell growth rate. As the change at the high dose was more than 10%, this test result was judged positive. This positive response was confirmed for the short-term and the continuous treatment without S9 mix at dose levels of 96.0, 137 and 196 µg/ml in each treatment. Under the condition of this assay, the Panel considers that DHC is clastogenic in the absence of metabolic activation.

An in vivo micronucleus study was carried out according to Ordinance No. 21 (March 26, 1997) on standards for conduct of non-clinical studies on safety of drugs, the Ministry of Health and Welfare, Japan (Nakajima, 2006; Bernard et al., 2008b). The assay was performed on male BDF1 mice (C57BL/6 x DBA/2) aged 9 weeks. Five animals per group were used. The test substance (DHC 95.8% pure) was diluted in MCT. The test substance was administered by gavage (0.5 ml/100g bw) at doses of 500, 1000 and 2000 mg/kg bw for 2 consecutive days. No significant decrease in the ratio of polychromatic erythrocytes (PCE) to the total number of analyzed erythrocytes was noted in any of the treatment groups. No statistically significant increase in the incidence of micronucleated

polychromatic erythrocytes (MNPCE) was noted in any of the treatment groups compared with the negative control. The Panel considers that under the conditions of this in vivo assay in mice, DHC was non-clastogenic.

An in vivo Comet assay in rats (Shimada, 2007; Bernard et al., 2008b) was carried out according to methodology described by Tice et al. (2003) and Hartmann et al. (2003). The assay was performed on male rats (Crl:CD(SD) aged 8 weeks. Four animals per group were used. The test substance (DHC content: 95.8%) was diluted in MCT. The test substance was administered daily by gavage (0.5 ml/100g bw) at the doses of 1000 and 2000 mg/kg bw for 2 consecutive days. At the end of treatment, the intestinal tract, liver and kidney were selected as the potential organs of absorption, metabolism and excretion for DHC. The tissues were taken 3 hours after the second dose. In the liver, in the DHC group treated with 2000 mg/kg bw, the increase in the Olive tail moment and the mean % tail DNA were slightly increased (1.72 and 1.57 times that of the negative control values, respectively) and was statistically significant ($p < 0.05$ Dunnetts Test); both parameters were within the range of variation of the historical control data for the testing facility. In the kidney, for the DHC group treated with 2000 mg/kg bw, the increase in the Olive tail moment was slightly increased (1.32 times that of the negative control value), and was statistically significant ($p < 0.05$). At 1000 mg/kg, the increase in the Olive tail moment and the mean % tail DNA were also slightly increased (1.35 and 1.21 times that of the negative control values, respectively), and was statistically significant using Dunnett's test at $p = < 0.05$). For the duodenum, the mean values in the Olive tail moment and the % tail DNA were statistically significantly ($p < 0.05$) increased at 2000 mg/kg bw being 1.72 times and 1.45 times that of negative control values, respectively). In the test groups, the actual values for Olive tail moment were lower than the historical data from the testing facility, and the mean values in the % tail DNA were almost the same as the historical data. Under the conditions of this assay, DHC showed equivocal evidence for DNA damage in rats at levels of 1000 mg/kg bw and 2000 mg/kg bw. The applicant argues that this result may have occurred because (1) the test has a high false positive rate due to apoptosis-induced DNA fragmentation or necrosis at levels inducing cytotoxicity (Tice et al, 2000; Olive et al, 1990); (2) the assay is known to be highly variable; (3) the increases fell within the historical control values of the testing facility and importantly (4) the increases that were observed were quantitatively very small, borderline or not dose-related. The Panel agrees with the applicant that the results of this assay are equivocal.

8.2.2. Genotoxicity tests on commercial grade DHC

A gene mutation assay of DHC in transgenic Big BlueTM rats (Nakajima, 2007a; Bernard et al., 2008b) was carried out according to the recommendation of the WHO-IPCS (2006). The assay was performed on male Big Blue™ rats aged 8 weeks. Five animals per group were dosed by gavage at 500 and 1000 mg/kg for 28 consecutive days. The test substance (DHC content: 94.0%) was diluted in MCT. After 3 days following the final treatment, the duodenum, liver and kidney were removed and analyzed for mutant frequency. No statistically significant increase in mutant frequency was observed in either the liver, the kidney or the duodenum of any groups. Under the conditions of this study, DHC was considered to be not mutagenic.

An in vivo micronucleus study (Nakajima, 2007b; Watanabe, 2008a) was carried out on DHC according to Ordinance No.21 (March 26, 1997) on standards for conduct of non-clinical studies on safety of drugs, the Ministry of Health and Welfare, Japan. The assay was performed on male BDF1 mice aged 9 weeks. Five animals per group were used. The test substance (DHCcontent: 94.0%) was diluted in MCT. DHC was administered by gavage at doses of 500, 1000 and 2000 mg/kg bw for 2 days. No significant decrease in the ratio of polychromatic erythrocytes (PCE) to the total number of analyzed erythrocytes was noted in any of the treatment groups. No statistically significant increase in the incidence of MNPCE was noted in any of the treatment groups compared with the negative control. The Panel considers that under the conditions of this in vivo assay in mice, DHC was non-clastogenic.

Summary of genotoxicity tests
The tests on laboratory scale DHC and commercial grade DHC are relevant for the assessment of genotoxic potential. The laboratory scale DHC was positive in vitro in the Ames-test (TA 100) as well as in the in vitro chromosomal abberation test; in both tests the positive result was seen only in the absence of S9 mix. The applicant argues that the latter indicates the ability for in vivo handling of potential genotoxicity of DHC.

Following these positive in vitro tests results, and in line with the EFSA Opinion on genotoxicity testing strategies (EFSA, 2011), appropriate in vivo testing for genotoxicity is recommended. For in vivo testing, it is necessary that evidence of target cell exposure be obtained in such studies. The Panel considers that the data on ADME (Section 8.1) are indicating the presence of DHC breakdown products in the blood stream, and are hence suggestive of exposure of the target tissues in vivo. EFSA (2011) also indicates that the Comet assay and the gene mutation assay with transgenic animals may be recommended as in vivo follow-up tests.

Both laboratory scale DHC and commercial grade DHC were negative in micronucleus tests in vivo. Laboratory scale DHC was equivocal in the Comet assay, and commercial grade DHC was negative in the gene mutation assay in transgenic rats.

The Panel considers that genotoxic activity of DHC observed in vitro in the presence of S9 mix is not expressed in vivo in mouse micronucleus studies with both laboratory scale DHC and commercial grade DHC, as well as in a gene mutation assay in transgenic rats with commercial grade DHC. Since commercial grade DHC is the product to be marketed the equivocal result of the in vivo Comet assay is considered less relevant for the assessment. Furthermore, there are no structural alerts for genotoxicity.

The Panel concludes that it has no safety concerns regarding genotoxicity.

8.3. Acute toxicity

An acute oral toxicity study (Kodama, 2007; Watanabe et al., 2008a) in mice was performed according to the respective Guideline in 'Redbook 2000 Guidance for industry and other stakeholders - Toxicological principles for the safety assessment of food ingredients'. Groups of 5 male and 5 female ICR mice (Crj:CD-1(ICR)) received the test substance (commercial grade; DHC content: 94.0 %) diluted in MCT at a dose of 5000 mg/kg bw (limit test). The control group received the vehicle alone. There were no deaths but during the two hour post-dose observation period staggering gait, prone position, decreased spontaneous movement, tremor, gasping or red-brownish urine were observed in some males and females in the group treated with DHC. These findings were transient and, with the exception of red-brownish urine, which was also noted after 6 hours post-dosing, there were no abnormal findings at later time points. No differences in body weights were observed between the test and control group, and there were no abnormal gross pathological findings at necropsy on day 15 after administration. The oral LD_{50} in mice was thus higher than 5000 mg/kg bw.

8.4. Subchronic toxicity

A 13-week subchronic oral toxicity study (Mochizuki, 2006; Kodama et al., 2008a) with laboratory scale DHC was performed according to the 'Guidelines for designation of food additives and for revision of standards for use of food additives', Notification No. 29 of the Environmental Health Bureau, Ministry of Health and Welfare, Japan (March 22, 1996). A GLP compliance statement was not provided. In this study, groups of 10 male and 10 female Sprague Dawley rats (Crl:CD(SD)) received the test substance (DHC content: 98.5 %) in MCT daily by gavage at doses of 100, 300 and 1000 mg/kg bw. The control group received the vehicle alone.

There were no deaths and no treatment-related clinical signs. No statistically significant differences were noted between the test groups and the control group regarding body weight, body weight gain, food consumption and water intake (except for a difference in water intake for females of the mid-dose group at one time point, which was not considered relevant). No abnormalities were detected in ophthalmoscopy. Haematology analysis showed no relevant differences between the treatment groups and the control group. Clinical-chemistry analysis showed significantly higher plasma alanine aminotransferase (ALT) activity (78 %) and total serum protein (TP) levels (7 %) in high-dose males. With regard to the increase in ALT activity, the applicant argued that the effect was solely due to a high value in a single male rat of the high-dose group. However, careful consideration of the individual data showed that two animals in the control group and one animal in the mid-dose group also had exceptionally high activities of specific enzymes (ALT, aspartate aminotransferase (AST) and lactate dehydrogenase (LDH)). After removal of these outliers the mean values for ALT activity were 22, 20, 23 and 34 IU/L for the control, low-, mid- and high-dose group, respectively (corresponding to an increase in high-dose males of approximately 41 % compared with the controls). Females at the highest dose level showed higher serum levels of calcium (5 %), TP (8 %) and albumin (5 %), a lower γ-globulin level (-18 %) and a higher albumin/globulin (A/G) ratio (9 %). Determination of organ weights at necropsy revealed increased liver weights, both absolute and relative, in high dose animals of both sexes (absolute weight: 16 % in males, 15 % in females; relative weight: 20 % in males, 11 % in females). Histopathological examinations of the liver and other selected organs and tissues showed no relevant differences regarding the incidence and severity of findings between animals of the high-dose group and animals of the control group.

The Panel considers that laboratory scale DHC at the highest dose level induced several changes which indicate an effect on the liver (i.e. higher ALT activity (males), higher albumin, lower γ-globulin levels, higher albumin/globulin (A/G) ratio and calcium levels (females), higher total protein levels and liver weights (both sexes)). The Panel considers that the mid-dose level, i.e. 300 mg/kg bw/day, is the no-observed adverse effect level (NOAEL) in this study.

Another 13-week subchronic oral toxicity study in rats (Ohishi, 2007; Watanabe, 2008b) was performed with commercial scale DHC in accordance with the 'Guidelines for designation of food additives and for revision of standards for use of food additives', Notification No. 29 of the Environmental Health Bureau, Ministry of Health and Welfare, Japan (March 22, 1996). A GLP compliance statement was not provided. Groups of 10 male and 10 female Sprague Dawley (Crl:CD(SD)) rats were administered the test substance (DHC content: 94.0 %) diluted in MCT by gavage at daily doses of 100, 300 and 1000 mg/kg bw. The control group received the vehicle alone.

No deaths or clinically relevant findings were noted during the treatment period. Food consumption in males of the high-dose group was consistently higher compared with the control group from week 42 onwards, reaching statistical significance at several time-points. This correlated with slightly, but not significantly, higher bodyweights in males of the high-dose group. High-dose males also showed increased water intake approximately from week 11 onwards, which was significant at several time-points. Ophthalmoscopy at the end of the treatment period did not reveal relevant changes. Haematology analysis showed no significant differences between the test groups and the control group. Clinical-chemistry analysis showed a significant increase in the plasma ALT activity in the high-dose group in both sexes (36 % in males; 29 % in females). In high-dose males, total serum protein (TP) levels were significantly increased and creatinine levels were decreased. Urinalysis showed an increase in urine volume as well as higher sodium, potassium and chloride concentrations in the urine for males of the high-dose group.

High-dose males showed a significant increase in absolute and relative liver and kidney weights (liver: absolute 19 %, relative 18 %; kidney: absolute 15 %, relative 12 %). High-dose females had higher relative liver and kidney weights (both 11 %). There were no relevant findings in the histopathological examinations of these organs with the exception of hepatocyte hypertrophy in two of the high-dose males (graded minimal in one rat and mild in the other) versus none in all other

groups. Examinations of other selected organs and tissues revealed no histopathological changes; the findings were randomly distributed between groups and thus considered incidental or spontaneous.

The changes observed in the high-dose group indicate an effect of the test material on the liver and kidneys. Therefore, the intermediate dose of 300 mg DHC/kg bw/day should be regarded as the no-observed adverse effect level (NOAEL) in this study.

The Panel notes that in this study commercial grade DHC at the highest dose level induced in part the same specific changes indicating an effect on the liver (i.e. increased ALT activity, total serum protein level and liver weights) as laboratory scale DHC, which was administered at the same dose level in the subchronic study described above. In this study, however, commercial grade DHC induced additional changes indicative of an effect on the kidney (i.e. changes in urinalysis parameters and higher kidney weights), which were not observed after administration of laboratory scale DHC. On request of the Panel to provide an explanation for these differences, the applicant considered differences in the production processes, the purity of the starting materials vanillyl alcohol (V-OH) and 8-methylnonanoic acid (MNA), and the specification of the tested materials. However, no cause for the observed differences in effects could be identified.

A 26-week oral toxicity study in rats (Kodama, et al 2008b; Ohishi, 2009) with commercial grade DHC followed by a 4-week recovery period was performed referring to the 'Guidelines for designation of food additives and for revision of standards for use of food additives', No. 29 of the Environmental Health Bureau, Ministry of Health and Welfare, Japan (March 22, 1996). No GLP compliance statement was provided. In this study, groups of 20 male and 20 female Sprague Dawley (Crl:CD(SD)) rats were administered the test substance (DHC content: 95.4 %) diluted in MCT daily by gavage at doses of 100, 300 and 1000 mg/kg bw/day for 26 weeks. The control group received the vehicle alone. Ten animals of each sex formed the recovery group and the respective control group.

There were no deaths during the treatment period but one high dose male was found moribund and killed on day 127. Necropsy showed a cerebellar nodule which, when followed by histopathology, was found to be a malignant oligodendroglioma in the cerebrum. This was judged to be a spontaneous finding unrelated to treatment. Two high-dose females with subcutaneous masses, one in the neck and one in the axilliary region, survived to term. The other animals showed no clinically relevant effects during the treatment period. There were no statistically significant differences in body weights between the test groups and the control group. Food consumption was higher in high-dose females compared to the control group from day 77 of treatment, the difference being statistically significant at several time points. Higher food consumption continued throughout the 4-week recovery period. Water consumption was higher in the high-dose group (statistically significant only in females at two time points) and remained higher in the high-dose females during the recovery period (significant at one time point). No abnormalities were identified in ophthalmological examinations.

Haematology analysis showed several significant differences between single test groups compared with the control group, the only notable one being higher mean platelet counts in male animals of the high-dose group, which persisted until the end of the recovery period. Regarding clinical-chemistry parameters ALT activity and total serum protein (TP) levels were significantly increased in the high-dose group in both sexes. Activity of ALT was also significantly higher in mid-dose males but the difference was less after removal of one outlier with an exceptionally high value. There were some shifts in specific serum protein fractions but the A/G ratio was not affected. Total cholesterol and phospholipid levels were increased in high-dose animals (significantly only in males, also at the mid dose). High-dose animals of both sexes had higher calcium levels, which according to the study report might be attributable to the increase in the level of the calcium-binding protein, albumin. At the end of the recovery period the only significant differences between the high-dose group and the controls were shifts in specific protein fractions and slightly, but not significantly, higher TP levels in both sexes. Activity of ALT in females was also still considerably higher compared with the controls (i.e. 48 vs. 19 IU/L after removal of outliers). Urinalysis after 3 months showed that high-dose males had

significantly higher urine volume (the water intake was not higher at this time point) compared with the controls. Urine volume in high-dose females was slightly, but not significantly, higher (water intake as well). After 6 months, urine volume as well as sodium, potassium and chloride concentrations in the urine were significantly higher in males (water intake not higher). Also at this time point, the pH value of the urine was slightly lower. Females also showed a slightly, but not significantly, higher urine volume (and water consumption) after 6 months. In both sexes a tendency towards decreased concentrations of ketone bodies was observed after 3 as well as 6 months. At the end of the recovery period females had a significantly higher urine volume (and water consumption but this was not significant). Also in males, urine volume (and water consumption) was still slightly, but not significantly, higher compared with the control group.

At necropsy after 6 months, absolute and relative liver weights were significantly increased in both sexes of the high-dose group. Other differences in the high-dose group concerned either absolute or relative weights, i.e. higher relative weights of the salivary gland, heart, spleen and kidneys in males and higher absolute weights of the pituitary gland (also significant in the mid-dose group), salivary gland, heart, lung and kidneys in females. After the recovery period, high-dose females had significantly increased relative liver and pituitary weights, and also salivary gland weights were slightly higher (not significant). Regarding absolute weights, pituitary gland, salivary gland and adrenal weights were significantly increased in females while liver and kidney weights were slightly higher (not significant). Histopathological examinations of the liver revealed a higher incidence of periportal hepatocyte hypertrophy compared with the controls in both sexes of the high-dose group (2 vs. 0 in males; 3 vs. 0 in females). High-dose females showed a higher incidence of tigroid cell foci (7 vs. 2; 5 and 1 per group were observed at the low- and mid-dose, respectively). Both sexes showed a higher incidence of hyaline urinary cast in the kidneys (males: 5 vs. 0; females 4 vs. 2). In females, tubular regeneration was increased in 7 animals (graded minimal) vs. 1 in the control group. In addition, higher incidences of extramedullary haematopoesis in the spleen (18 vs. 14) and glandular stomach erosion (6 vs. 3) were observed in females. The incidence of focal acinar cell atrophy in the pancreas was higher in males (3 vs. 0). After the recovery period there was still a higher incidence of tubular regeneration (3 vs. 0) in female kidneys, and males also showed a difference at that time point (7 vs. 4). The presence of hyaline urinary cast in female kidneys was still slightly higher (3 vs. 1). Adenocarcinoma (2 vs. 0) and increased incidence of focal and lobular hyperplasia (2 vs. 0) were observed in females of the high-dose group at the end of the recovery period but the observed incidences were within the normal range for female rats of this strain and age (historical control data were provided). There was also one animal each in the control and the high-dose group with mammary adenocarcinoma after the 6 month treatment period.

In this 26-week oral toxicity study, commercial grade DHC induced similar effects as those observed in the 13-week rat study using the respective test material. As in the shorter study, the changes in specific parameters, which indicate an effect on the liver and kidneys, occurred only at the highest dose level. Furthermore, in this study, slightly higher incidences of histopathological changes in the liver and kidneys compared with the controls were identified in the high-dose group, which was not the case in the study of shorter duration. The study also showed that not all effects induced by the test material were reversed at the end of a 4-week recovery period. Therefore, the Panel is of the opinion that the mid-dose level, i.e. 300 mg DHC/kg bw/day, should be regarded as the NOAEL in this study.

Studies on chronic toxicity/carcinogenicity were not provided.

8.5. Reproductive and developmental toxicity

A developmental toxicity study (Ikeya, 2007; Bernard, 2008c) with commercial grade DHC administered orally to rats was performed according to the 'Guidelines for designation of food additives and for revision of standards for use of food additives', Notification No. 29 of the Environmental Health Bureau, Ministry of Health and Welfare, Japan (March 22, 1996). A GLP

compliance statement was not provided. Groups of 18-20 female Sprague Dawley (Crl:CD(SD)) rats received the test substance (DHC content: 94.0 %) diluted in MCT by gavage at doses of 100, 300 and 1000 mg/kg bw/day. The control group received the vehicle alone. Animals mated at 11 or 12 weeks of age were dosed for 11 consecutive days on days 7-17 of gestation. Dams were killed on day 20 of gestation. There were no deaths and no differences between the test groups and the control group regarding general condition, body weight development and food consumption of the dams. No pathological changes were noted in the macroscopic examination at necropsy. Examination of uteri contents revealed no relevant differences in the number of corpora lutea, the number of implantations, the implantation index, the index of resorbed/dead foetuses and the sex ratio of live foetuses. No external malformations were noted regarding live foetuses and the placentas appeared normal. Body weights of male foetuses in the high-dose group were significantly higher than in the control group but, according to the study report, within the normal ranges. Visceral and skeletal examinations of the foetuses revealed no relevant differences in the incidences of abnormalities and variations between the groups. There were no relevant differences regarding the progress of ossification. It is concluded that administration of DHC had no adverse effects on pregnant rats or on foetal growth and development. Thus, the NOAEL in this study is 1000 mg/kg bw, the highest dose administered.

In a similarly designed study (Matsuoka, 2007; Bernard et al., 2008c), which was performed according to the same Guideline (GLP certificate not provided), commercial grade DHC was administered to rabbits. Groups of 22 pregnant New Zealand White rabbits (Kbl:NZW) received the test substance (DHC conent: 94.0 %) diluted in MCT by gavage at doses of 100, 300 and 1000 mg/kg bw. The control group received the vehicle alone. Animals mated at 16-17 weeks of age were dosed for 13 consecutive days on days 6-18 of gestation. The dams were killed on day 28 of gestation.

Regarding the dams, there were no deaths, clinically relevant effects or abortions. Body weights and body weight gains in the test groups did not differ significantly from the control group and food consumption was comparable in all groups. At necropsy, no relevant gross pathological findings were noted. Examination of uteri contents revealed no significant differences in the number of corpora lutea, the number of implantations, the implantation index and the index of resorbed/dead foetuses. No external malformations were noted regarding live foetuses and the placentas appeared normal. There were no differences in sex ratio and foetal body weights between the test groups and the control group. No relevant differences in the incidence of visceral and skeletal abnormalities, and in variations in foetuses, were identified between the groups. There were also no relevant differences regarding the progress of ossification. It is thus concluded that administration of DHC had no adverse effects on pregnant rats or foetal growth and development. The highest dose administered, i.e. 1000 mg/kg bw/day, is regarded as the NOAEL in this study.

Studies on reproductive toxicity with DHC were not provided.

8.6. Human studies

A placebo-controlled, randomised, double-blind study with parallel design was performed with 34 healthy male Japanese subjects (age 30-65 years). In this study, DHC was administered in capsule form at doses of 3 or 12 mg/day for 8 days (Kajimoto, 2009a). The control group received the placebo (vehicle only; rapeseed oil). On the first and last day of the study the participants completed a questionnaire with regard to the experience of symptoms, and blood samples were taken for clinical-chemistry and haematology examinations. The administration of DHC was well-tolerated and none of the subjects discontinued dosing due to subjective symptoms or adverse events. The overall incidence of subjective symptoms and abnormal laboratory data was similar in the placebo group (3/11), DHC low-dose group (1/12) and high-dose group (2/12). Symptoms were recorded in 5 subjects, and the reported findings included general malaise (n=1), cough and nasal discharge (n=1), and sore throat (n=1) in the placebo group, constipation as well as bradycardia, pallor, cold sweat and blood pressure fall (n=1) in the low-dose group and stiff shoulders (n=1) in the high-dose group. One additional

subject in the high-dose group showed higher cholesterol and blood urea nitrogen (BUN) levels at the end of the treatment period. Regarding clinical-chemistry and haematology analyses, as well as blood pressure measurements, there were no clinically relevant differences between the placebo and DHC groups at baseline (pre-treatment) or at the end of the treatment period. There were also no clinically relevant differences between the baseline and final situation in each group.

In a placebo-controlled, randomised, double-blind study with parallel design including 70 healthy male Japanese subjects (age 20-59 years), DHC was administered in beverages at doses of 3 mg/day (n=23) or 9 mg/day (n=23) for 4 weeks (Kajimoto, 2009b). The control group (n=24) received a placebo (beverage without DHC). On the first and last day of the treatment period the participants completed a questionnaire with regard to the experience of symptoms, and blood samples were taken for clinical-chemistry and haematology examinations. DHC was well-tolerated in this study and none of the subjects discontinued dosing due to adverse events. The overall incidence of subjective symptoms was similar in the placebo group (4/24), the DHC low-dose group (2/23) and DHC high-dose group (3/23). The most common findings were headache (n=1 in the placebo group and n=2 in the high-dose DHC group) and rhinitis (n=1, high-dose). Other signs included heartburn (n=1, high-dose), sore throat (n=1, low-dose), loose stool (n=1, low-dose), dermatitis (n=1 placebo), atopic dermatitis (n=1 placebo) and a decrease in body weight (n=1, placebo). There were statistically significant differences between day 0 (pre-treatment, baseline) and day 28 (post-treatment) in several clinical-chemistry and haematology parameters in all groups, and there were some statistically significant differences between the placebo and DHC groups on day 0 as well as on day 28. However, the variations were assessed to be of no clinical relevance since the changes were within the normal range of biological variation. Also, no clinically relevant changes in blood pressure were noted.

In conclusion, administration of 3 mg or 9 mg DHC/day administered in a beverage over 4 weeks was found to be well tolerated. The dosages equated to approximately 0.05 mg/kg bw/day and 0.15 mg/kg bw/day based on a bodyweight of 60 kg.

The Panel is of the opinion that the human studies are of little relevance for the safety assessment since they were of short duration, the administered doses were low, and the number of safety endpoints studied was limited.

Interaction with human vanilloid type-1 (TRPV-1) receptors

Capsaicin exerts its pharmacological effects through the capsaicin receptor, known as the vanilloid receptor (VR1) or transient receptor potential vanilloid subfamily member (TRPV1). TRPV1 is a non-selective cation channel activated by a wide range of physical and chemical stimuli. Similarly to capsaicin, capsinoids can activate TRPV1 (Luo et al., 2011). Dihydrocapsiate, capsiate and nordihydrocapsiate bound to TRPV1 receptors expressed in cultured cells activated Ca2+ influx in a concentration-dependent manner with similar magnitudes; the potency of the capsinoids was approximately 1/10 that of capsaicin (Sasahara et al., 2010).

Pharmacokinetic studies in rats (Bernard et al., 2010), as well as the results of a human study (Bernard et al., 2008), suggest that DHC is hydrolysed, followed by rapid absorption of the metabolites, conversion into conjugates and excretion. In contrast to the more stable capsaicin, dihydrocapsiate is not expected to enter into the systemic circulation in intact form. Effects, such as the activation of the sympathetic nervous system (Hachiya et al., 2007), are considered to be due to the activation of TRPV1 expressed on the GI tract surface.

There are no data indicating that the hydrolysis products vanillyl alcohol and 8-methyl-nonanoic acid show specific interactions with TRPV-1 receptors.

Allergenicity

No allergenic reactions have been reported in workers involved in the production of DHC or CH-19 Sweet extract which is marketed as a food supplement in Japan since 2006 and in the USA since 2007.

As dihydrocapsiate is synthesised and not extracted from plant material it is unlikely to cause IgE food related allergy. The only potential source of protein entering the production process would be from the lipase enzyme in the esterification reaction. The enzyme is immobilised in an inert carrier and cannot partition into the n-hexane fraction containing DHC. In the worst case scenario, even if granulate "fines" containing the immobilised enzyme entered the hexane layer, the particles would be trapped during filtration (filter porosity is 5 μm) and would be separated from the DHC product. The applicant states that the enzyme manufacturer (Novozymes, Denmark) has conducted studies to illustrate that the carrier is robust under normal usage, and that there is no release of the enzyme or other materials.

Although no studies on allergenicity were provided by the applicant, the Panel considers that it is unlikely that the novel ingredient poses an allergenic risk.

DISCUSSION

Considering the proposed uses the estimated mean intake of synthetic DHC was estimated to be around 12 – 13 mg/day (8.1 mg/day for pre-school children); the 97.5th percentile intakes of adults and the elderly were estimated to be around 34 mg/day (18.5 mg/day for pre-school children) based on UK consumption data. Calculations based on body weights resulted in the highest intakes being for pre-school children (mean: 0.6 mg/kg bw/day; 97.5th percentile: 1.3 mg/kg bw/day).

The available data on absorption, distribution, metabolism and excretion (ADME) suggest that ingested DHC is rapidly metabolised in the gut of rats and humans to form vanillyl alcohol, vanillic acid and 8-methylnonanoic acid. After absorption the first two are converted into glucuronide and/or sulphate conjugates in the liver, and eliminated predominantly by the kidneys into the urine. Total excretion in the urine, faeces and expired air after 72 hr was 98.0 %. The methyl-branched fatty acid 8-methylnonanoic acid is likely to be subject to degradation via ß-oxidation. Terminal ω-oxidation might constitute an alternative pathway.

The applicant has provided a range of toxicological studies with DHC, including in vitro and in vivo genotoxicity studies. Based on the results of these studies and in the absence of structural alerts for genotoxicity, the EFSA NDA Panel concludes that it has no safety concerns regarding genotoxicity. Studies on developmental toxicity in rats and rabbits using commercial grade DHC did not show adverse effects on pregnant animals or on foetal growth and development up to the highest dose administered (1000 mg/kg bw/day). In a 13-week oral toxicity study in rats with laboratory scale DHC, several changes were observed at the highest dose level (1000 mg/kg bw/day) which indicate an effect on the liver. Administration of commercial grade DHC to rats for 13 weeks at the same dose level induced in part the same specific changes indicating an effect on the liver. In addition, commercial grade DHC induced additional changes indicative of an effect on the kidneys. No explanation for the observed differences could be provided by the applicant. The Panel concludes that the intermediate dose of 300 mg DHC/kg bw/day is the NOAEL in these 13-week studies. In a 26-week oral toxicity study, commercial grade DHC induced at the highest dose level (1000 mg/kg bw/day) similar changes regarding liver- and kidney-specific parameters to those observed in the 13-week study with commercial grade DHC. Furthermore, slightly higher incidences of histopathological changes in the liver and kidneys compared with the controls were identified in the high-dose group. Not all effects induced by the test material were reversed at the end of a 4-week recovery period. The Panel concludes that 300 mg DHC/kg bw/day is the NOAEL in this study.

Taking account of the estimated intake levels resulting from the uses as proposed by the applicant, the Panel is of the opinion that the margin of safety in relation to the NOAEL of 300 mg/kg bw/day in the 26-week rat toxicity study is sufficient, including the highest estimated intake of 1.3 mg/kg bw/day for preschool children.

CONCLUSIONS

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

DOCUMENTATION PROVIDED TO EFSA

1. Dossier "Dihydrocapsiate" received on 5 December 2011. Submitted by Ajinomoto Co. Inc. Additional data were provided on 14 May 2012.
2. Letter from the European Commission to the European Food Safety Authority with the request for an opinion on the safety of 'Dihydrocapsiate'. Ref. Ares (2011)1197735, received on 10 November 2011.
3. Initial assessment report carried out by the United Kingdom, 'Dihydrocapsiate' as novel food ingredients, Initial assessment under Article 4 of Regulation (EC) No 258/97".
4. Member States' comments and objections.
5. Response by the applicant to the initial assessment report and the Member States' comments and objections.

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