Food Standards Agency
food.gov.uk

Mr Andreas Klepsch
European Commission
By Email

10 March 2011

Dear Mr Klepsch

INITIAL OPINION:SYNTHETIC DIHYDROCAPSIATE AS A NOVEL FOOD INGREDIENT.

On 6 August 2010, the UK Competent Authority accepted an application from Ajinomoto Co. Inc. for authorisation of synthetic dihydrocapsiate (DHC) as a novel ingredient in accordance with Article 4 of Regulation (EC) 258/97.

The Advisory Committee on Novel Foods and Processes (ACNFP) reviewed this application and their opinion is attached.

In view of the ACNFP's opinion, the UK Competent Authority considers that DHC, for use in the foods proposed by the applicant at levels of 3 mg/serving or portion meets the criteria for acceptance of a novel food, as set out in Article 3 (1) of Regulation 258/97.

Yours sincerely,

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ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

OPINION ON AN APPLICATION UNDER THE NOVEL FOODS REGULATION FOR SYNTHETIC DIHYDROCAPSIATE

Applicant:	**Ajinomoto Co. Inc.**
Responsible Person:	**Andrew Cockburn**
EC Classification:	**1.2**

Introduction

1. An application was submitted to the Food Standards Agency in August 2010 by Ajinomoto Co. Inc for the authorisation of synthetic dihydrocapsiate (DHC) as a novel ingredient in the EU. A copy of the application was placed on the Agency's website for public consultation.

2. Dihydrocapsiate was first discovered in CH-19 Sweet (a non-pungent variety of chilli pepper) along with capsiate. Sourcing of large quantities of dihydrocapsiate from chilli peppers is not sustainable because of the relatively small amounts that are contained in and can be extracted from chilli peppers.

3. Both dihydrocapsiate and capsiate occur naturally in edible pungent (hot) and non-pungent chilli peppers. They are analogues of capsaicin, the pungent component of chilli peppers, but unlike capsaicin they do not create the sensation of "hotness". Dihydrocapsiate and capsiate have an ester bond in place of the amide bond of capsaicin between the vanillyl and fatty acid moieties.

4. The applicant mentions in the dossier that capsinoids are able to enhance energy expenditure and fat oxidation.

5. The applicant reports that an extract of capsinoids from CH-19 Sweet chilli pepper is marketed as a food supplement in the EU (Czech Republic and France) and in the US and Japan.

6. The applicant intends that synthetic dihydrocapsiate (DHC) will be incorporated 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.

7. DHC has been classified as a pure chemical or simple mixture from non-GM sources where the source of the novel food has no history of food use in the EU (class 1.2 accoridng to the scheme in Commission Recommendation 97/618 (EC)).

I. Specification of the novel food

Information on this aspect is provided on p. 10-15 of the application dossier

8. The chemical and physical specification for DHC has been established by the applicant and can be found in the table below.

Test Item	Test Method	Acceptance Criteria
Description	JSFA V11, general notices	Viscous, colourless to yellow liquid
Identification (IR)	FCC V, Infrared Spectra	It exhibits absorption at the wave number of around 2953, 2928, 2855, 1733, 1519, 1278, 1241, 1036, 818 and 798 cm^{-1}
Specific Gravity	FCC V, Specific Gravity	1.02 to 1.03
Starting Materials	HPLC	Vanillyl alcohol: not more than 1.0% MNA*2% to 7%
Related Substances	HPLC	Not more than 2%
Residual Solvent (n-hexane)	GC	Not more than 5 mg/kg
Assay (DHC)	HPLC	≥ 94 %
Magnesium	JSFA VII, Atomic Absorption Spectrophotometry	Not more than 1 mg/kg
Copper	JSFA V11, Atomic Absorption Spectrophotometry	Not more than 1 mg/kg
Arsenic	JP XIV, Arsenic Limit Test, Method 4	Not more than 1 mg/kg
Cadmium	FCC V, Flame Atomic Absorption Spectrophotometric Method	Not more than 1 mg/kg
Lead	FCC V, Lead Limit Test, Flame Atomic Absorption Spectrophotometry	Not more than 1 mg/kg

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
GC: Gas Chromatography
HPLC: High-Performance Liquid Chromatography
*MNA = 8-methylnonanoic acid

9. The applicant has provided results of analysis of seven independently manufactured lots of commercial grade DHC produced on a pilot scale over a three month period which demonstrates that all lots conformed with the set specifications. The applicant states that it was demonstrated over this period that the manufacturing process and final product are highly reproducible and that the process is capable of consistently producing material that meets the above specifications. The analysed batches were produced on a pilot scale but using the same type of industrial equipment so the applicant states it is therefore reasonable to expect that scaling up of the process will not result in changes to the composition of DHC preparations.

10. The product contains a minimum of 94% DHC and the impurities have also been characterised. Analyses of the same seven batches revealed the presence of reaction products or related substances which comprised between 0.69 and 1.39% of the product. The applicant has identified four major by-products which accounted for 77 to 91% of total other related substances, namely vanillyl 6-bromohexanoate, vanillyl decanoate, vanillyl dihydrocapsiate and a diacyl ester. Of these, vanillyl dihydrocapsiate was the largest individual impurity with a concentration of 0.73%.

11. Residues of the extraction solvent n-hexane are kept to specifications (less than 5 parts per million).

***Discussion**: The Committee did not raise any concerns relating to this section of the dossier.*

II. Effect of the production process applied to the novel food

Information on this aspect is provided on p 16-20 of the application dossier

12. DHC is produced by esterification of vanillyl alcohol (V-OH) and 8-methylnonanoic acid (MNA) using an immobilised food grade lipase preparation. The lipase enzyme is produced by Novozymes Denmark (Novozyme®435 FG, declared activity 1000PLU/g) and according to the applicant is approved in the EU as a processing aid (approval reference number 2006-20-5406-00106).

13. After esterification, the reaction is quenched with the addition of n-hexane. The lipase and V-OH are removed by filtration steps.

14. The applicant has presented details of stability studies which show that DHC is stable for at least 2 years at either 5°C or 20°C. The applicant has determined the shelf-life of the product to be 12 months (minimum).

***Discussion**: The Committee did not raise any concerns relating to this section of the dossier.*

IX. Anticipated intake/extent of use of the novel food

Information on this aspect is provided on p 36-42 of the application dossier

15. The applicant plans to produce DHC for use by third party food manufacturers but will not itself manufacture foods containing DHC. The applicant intends that DHC will be incorporated 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. Therefore, the applicant is seeking approval for use of DHC in these foods at levels that will deliver 3 mg per portion or serving. The proposed use levels have been calculated so that each portion of a given food product will contain 3 mg of DHC. The actual DHC concentration in any food will therefore depend on that manufacturer's product specification for single serve products or on the typical or recommended portion sizes for products presented in multi-serve packs.

16. Individual intake of DHC will depend on how many servings of food containing 3 mg of DHC are consumed. The applicant has provided a detailed section on potential intakes in the dossier. The applicant anticipates that DHC-containing foods will normally be consumed by adults, but has calculated intakes for all potential consumers.

17. The applicant estimates that, based on UK and European food consumption patterns, average adult intakes are not likely to exceed 25 mg/day (0.4 mg/kg bw/day) and high level intakes are not likely to exceed 40 mg/day (0.7 mg/kg bw/day). These estimates are based on the conservative assumption that all possible foods in an individual's diet contain 3mg DHC per portion. The applicant further states that if children were to consume DHC in all foods which could potentially contain it, their average intake could be up to 15 mg/day (1 mg/kg bw/day) and high level intake may approach 30 mg/day (2 mg/kg bw/day). The applicant indicates that dihydrocapsiate from natural sources is unlikely to contribute significantly to European intakes so these sources have not been included in these calculations.

***Discussion**: The Committee raised a question relating to intake estimation in view of the broad range of products to which DHC is intended to be added. Exposure specialists in the Food Standards Agency advised that the applicant's approach using NDNS data and the EFSA concise database was*

reasonable, particularly as the modelling was done on a wide range of food groups. Therefore, no further information was requested from the applicant on this issue. Setting levels on the basis of portion sizes has been used in previous novel food applications where an applicant has provided information about the use level required to achieve a particular level of intake, based on typical portion sizes. The Committee did not raise any further questions relating to this issue.

XI. Nutritional information on the novel food

Information on this aspect is provided on p 43 of the application dossier

18. The applicant states that synthetic DHC is identical to dihydrocapsiate found naturally in peppers and can therefore be considered to be nutritionally equivalent to the natural product and likewise has negligible nutritional value.

***Discussion**: The Committee sought clarification of the purpose of adding DHC to foods. The applicant highlighted that chillies can have desirable properties for consumers e.g. providing a feeling of refreshment or well being. The addition of DHC is intended to provide the same response but without the strong hot taste. The Committee remained sceptical about the purpose of incorporating DHC into foods but this was not a safety-related concern.*

XII. Microbiological information on the novel food

Information on this aspect is provided on p.15 and p44 of the application dossier

19. The applicant has provided microbiological data from 4 independent lots of DHC, where total aerobic counts were <3000 CFU/g, yeast and mould counts were <100 CFU/g and all lots were negative for Coliforms.

***Discussion**: The Committee did not raise any concerns or questions on this aspect of the application.*

XIII. Toxicological information on the novel food

Information on this aspect is provided on p. 45-75 of the application dossier

<u>Metabolic fate of DHC</u>

20. The applicant states that, based on toxicokinetics data in rats, the highest concentrations of DHC after oral dosage are found in the major organs or systems of absorption, metabolism and excretion (the GI tract, liver and kidney). DHC is metabolised by hydrolysis in the gut and the metabolites are rapidly absorbed and conjugated in the liver and eliminated predominantly by the kidneys into the urine. The applicant concludes that

accumulation of DHC or its metabolites in the tissues is unlikely to occur due to its rapid absorption, short half-life and high level of excretion.

21. The applicant has also summarised relevant studies on the metabolic fate of CH-19 Sweet extract (which contains ca. 7.5% capsinoids, around 20% of which is dihydrocapsiate). These studies showed that capsinoids from CH-19 sweet extract are metabolised in the GI tract and or gut mucosa (or both) before absorption. The absorbed vanillyl alcohol found in the portal vein undergoes metabolic conversion by sulphation and glucuronidation during its passage through the liver before entering into the post-hepatic systemic blood. Rapid and extensive absorption and metabolism occurs following oral administration to rats and man. The applicant states that there was no evidence for inhibitory effects on the mixed function oxidase CYP3A4.

22. The applicant states that DHC cannot be accumulated in adipose tissue or the brain as it is readily metabolised to VOH and 8-MNA in the GI tract and is not itself absorbed systemically (the applicant has cited a recent study relating to this). The applicant has outlined a study (Bernard *et al*., 2010) investigating the tissue distribution of a single oral dose (10 mg/kg bw) of ^{14}C-DHC in fasting rats together with the metabolic profiles for DHC both before and after enzymatic hydrolysis. Radioactivity was measured in the tissues for up to 24 hours. The applicant states that the results show that DHC is metabolised in a multistep process, initially to VOH and 8-MNA. Subsequently, the majority of VOH is conjugated to glucuronate or sulphate, with minor amounts oxidised to vanillic acid. The applicant states that because of the rapid and apparently complete breakdown of DHC in the GI tract, the observed systemic radioactivity came from the VOH metabolites/conjugates, due to radio-labelling of DHC.

23. Based on these study results, the applicant states that radioactivity in the brain (cerebrum) was below the limit of detection 24 hours after dosing so VOH apparently did not accumulate in the brain. Radioactivity was observed until 24 hours after dosing in adipose tissue (fat and brown fat) but the level decreased in parallel with that in the plasma (plasma half life was calculated to be 2.4 hours). Plasma T_{max} was achieved 40 minutes post dosing and the radioactivity declined thereafter. The applicant states that total excretion after 72 hours was 98.1% (78.2% urine, 19.4% faeces and 0.5% expired air). Residual radioactivity in the carcass (including GI tract and gall bladder) was 4% of the dose at 72 hours. The applicant acknowledges that the metabolism of 8-MNA was not investigated in this study due to the position of the radiolabel but the applicant states that as 8-MNA is a mid-chain fatty acid, it is likely to be metabolised by mitochondrial or peroxisomal ß oxidation.

Toxicology – Animal and *in vitro* studies

24. The applicant has provided details of animal studies conducted on commercial grade DHC (the novel ingredient in the form to be marketed), laboratory scale DHC (an earlier form of the novel ingredient prior to scaling up) and CH-19 Sweet extract. The applicant has provided data to demonstrate that commercial grade DHC and the laboratory scale version complied with the specifications for DHC. The applicant has also provided specifications for CH-19 Sweet extract and shown that the Lots used as toxicological test material comply with these specifications.

25. Animal studies on commercial grade DHC are presented in the dossier. The applicant has provided details of 13 week and 26 week rat studies in addition to teratology and developmental toxicity studies in rats and rabbits. The applicant states that based on results from studies, DHC has a low acute oral toxicity (>5 g/kg), is well tolerated on repeat dose administration over 13 or 26 weeks by oral gavage, is non teratogenic and non-mutagenic or clastogenic in *in vivo* studies. The applicant concludes that these studies demonstrate an overall NOAEL of 1000 mg/kg bw/day. The only consistent changes observed were in the subacute and subchronic rat studies where slight weight increases in the liver and kidney were observed at the 1000 mg/kg dose, but in the absence of toxicity as evidenced by histopathological examination. The applicant acknowledges that small changes in alanine transaminase (ALT) were observed in individual animals but states that these were generally within normal limits for the age and sex of rats involved and for the contract testing facility. The applicant states that minimal or mild grade hepatocellular hypertrophy was seen in two high dose level male rats in the 13 week study, but was not seen in the 26 week study. In consequence, the high dose level in both the 13 week and 26 week studies was judged not to be toxic, thus providing a NOAEL of 1000 mg/kg. This NOAEL is 1300 times higher than the estimated high level intake by adult consumers (see paragraph 17 above).

26. The applicant has also provided data from gene mutation and mouse micronucleus studies and states that based on these studies DHC is not mutagenic or clastogenic.

27. The applicant has also provided details of toxicity studies conducted on laboratory scale DHC. The studies and results are summarised in the following table.

Study	Author, study experiment No.	Result
13-week oral gavage toxicity study in rats.	Mochizuki, M. 2006 N-B205	NOAEL >1000 mg/kg
Bacterial Reverse mutation test	Shimada, S. 2006 9612 (258-046)	Non mutagenic +S9, mutagenic in TA100 only in absence of S9.
In vitro chromosome aberration test	Masumori S. 2006 9613 (258-047)	Non-clastogenic +S9. Clastogenic only in absence of S9.
In vivo mouse micronucleus test	Nakajima M. 2006 9623 (258-048)	Non-clastogenic
In vivo Comet assay in rats	Shimada S. 2007 9993 (258-061)	Equivocal DNA damage, within historical control values.

28. As some of the toxicological studies revealed unexpected results relating to genotoxicity, the applicant carried out relevant follow-up *in vivo* studies which yielded negative results.

29. For completeness, the applicant has provided details of toxicity studies conducted on CH-19 Sweet extract, which contains 7.5% capsinoids of which ca. 20% is dihydrocapsiate. The level of dihydrocapsiate is identified for each study and a summary table to these toxicity studies is provided below:

Study	Dihydrocapsiate dose equivalent (mg/kg)	Author, Study/experiment number	Result (mg/kg Dihydrocapsiate)
Single dose acute oral toxicity tests in rats.	71.25 142.50 285	Mochizuki M. 2005	LD50 >285 mg/kg
13-week oral gavage toxicity study in rats	Low 16.63-20.19 Mid 33.25-40.38 High 66.50-80.75	Mochizuki, M. 2006a.	NOAEL 66.5 to 80.75*
26 week oral gavage toxicity study in rats	Low 16.63-20.19 Mid 33.25-40.38 High 66.50-80.75	Mochizuki, M. 2006b	NOAEL in males 33.25 to 40.38* NOAEL in females 66.5 to 80.75*

Oral gavage teratology and developmental toxicity study in rats	Low 20.19 Mid 40.38 High 80.75	Katsumata Y. 2006a N-R013	Maternal and foetal NOAEL 80.75
Oral gavage developmental toxicity study in rabbits	Low 3.8 Mid 7.6 High 15.2	Matsouka T. 2006 N-R010	Maternal and foetal NOAEL 15.2
Two generation oral gavage reproduction study in rats	14.25-20.19 28.5-40.38 57-80.75	Katsumata Y. 2006b N-R008	NOAEL 57 to 80.75*
Bacterial reverse mutation test	-	Nakajima. 2005 9224 (258-041)	Not mutagenic
In vitro chromosome aberration test	-	Masumori S. 2005a 9225 (258-042)	Not clastogenic
Mouse micronucleus test	-	Masumori S. 2005b 9226 (258-043)	Not clastogenic

*due to range of DHC content in different Lots of CH-19 Sweet extract

- plate concentration conversion in DHC equivalent not calculated.

Human studies

30. The applicant presented two human studies which showed that DHC administered in capsules at 3 or 12 mg volunteer/day for 8 days or in beverages at 3 or 9 mg volunteer/day for 4 weeks, was well tolerated and gave rise to no obvious dose related clinical signs or treatment related effects. The applicant stated that the occasional and sporadic findings recorded in the two separate studies seldom occurred in more than one volunteer/sign and there was no consistent pattern or trend. The side effects reported in the studies included stiff neck, high total cholesterol and blood urea nitrogen, constipation, bradycardia and loose stools. These studies also revealed some variations in blood pressure in DHC-treated individuals but the applicant concluded that these minor variations are

unlikely to be related to treatment as increases were observed in one study and reductions in the other.

31. The Committee examined detailed reports of the human studies in relation to the apparent blood pressure-related changes that were observed and agreed that these were not a cause for concern.

32. The Committee requested additional information from the applicant on pharmacological and nutraceutical effects of DHC or its metabolites. Although efficacy assessment of a novel ingredient is not within the remit of the Committee's function, in this instance, Members felt this information would be useful in evaluating the safety of the product. The Committee was particularly interested in the interaction of DHC or its metabolites with vanilloid receptors in the mouth and gut and whether DHC or its metabolites may give rise to any cardiovascular or neurological effects.

33. The applicant explained that DHC interacts with vanilloid type-1 (TRPV-1) receptors on the tongue and provided background information on this family of proteins. TRPV-1 is a protein which is a member of the TRPV group of transient receptor potential family of ion channels and in humans is encoded by the TRPV-1 gene. TRPV-1 is a non-selective ion channel and may be activated by a wide range of exogenous and endogenous stimuli, the best known being heat greater than 43°C and capsaicin. The applicant states that both capsaicin and capsinoids interact with and activate TRPV-1 receptors in the same manner but the potential for interaction of DHC with oral TRPV-1 receptors is less than that for capsaicin.

34. The applicant also stated that DHC acts locally in the GI tract to activate TRPV-1 receptors. These receptors are expressed on the peripheral terminals of the primary sensory neurons such as the vagus nerve. The applicant cites a study to show that DHC is rapidly metabolised to vanillyl alcohol (VOH) and 8-methyl-nonanoic acid (8-MNA) in the GI tract and intact DHC is not absorbed in the systemic circulation. The applicant states that while both of these DHC metabolites are absorbed, neither is expected to have significant pharmacological activity or have any specific interaction with TRPV-1 receptors. The applicant states that there is no evidence to suggest that DHC can give rise to cardiovascular effects and has highlighted specific studies to reiterate this point. One study highlighted that a single oral dose of up to 30 mg of capsinoids (approx. 8 mg DHC) per person did not result in any increase in blood pressure/heart rate or any other clinically relevant effects in healthy volunteers. In another study, capsaicin supplementation (150 mg/person) one hour before exercise intervention had no effect on cardiac autonomic nervous system activities

and cardiac electrical stability during exercise in obese individuals (the applicant states these findings can read across to DHC because it interacts with TRPV-1 receptors in the same way as capsinoids). A final study highlighted by the applicant to demonstrate the lack of any generalised response that could lead to cardiac involvement revealed that there were no significant changes in the levels of either plasma or urine catecholamines (adrenalin and nor-adrenalin) after ingestion of 30 mg/person of capsinoids (approx. 8 mg DHC/person). Catecholamine levels were measured at 15 and 30 minutes after ingestion, then again at 1, 2, 4, 8 and 24 hours for plasma and at 24 hours after ingestion for urine.

35. The applicant states that DHC only has local sensory effects and these are mediated via the TRPV-1 receptors on the surface of the GI tract, from the buccal cavity along the length of the gut. The applicant does however state that local activation of TRPV-1 receptors by DHC can impact both brown and white adipose sympathetic receptors through stimulation of the vagus afferent nerve and the sympathetic nervous system but not the heart.

***Discussion**: The Committee noted that the applicant had derived a NOAEL of 1000 mg/kg from the animal feeding studies conducted with DHC. The Committee considered that a NOAEL of 300 mg/kg would be more appropriate, but emphasised that a large safety margin still exists at the 300 mg/kg level.*

The Committee did not have any significant safety concerns relating to DHC. The Committee noted that DHC interacts in different ways with TRPV1 receptors along the GI tract and no further information was requested from the applicant on this issue.

The Committee requested further clarification of the applicant's statement that the metabolites of DHC (8-MNA and VOH) are not expected to have any pharmacological activity. The applicant explained that both metabolites have food uses[1] and no references have been found in the literature to indicate any significant intrinsic pharmacological activity. The applicant additionally stated that, taking into account the long history of safe use observed following natural systemic exposure to both substances from the traditional consumption of chilli peppers and, in the case of VOH, from vanilla (vanillin), together with the lack of any apparent pharmacological effects in the animal toxicology and human clinical trials with DHC there is an overall lack of evidence for any significant pharmacological activity of these two metabolites following oral administration. This comment excludes flavour as a 'pharmacological effect. Thus, while both

[1] VOH is a permitted food flavouring in the EU. 8-MNA is designated as a flavouring in Japan by the Ministry of Health, Labour and Welfare.

are absorbed, neither metabolite is expected to have any specific interaction with TRPV-1 receptors.

The Committee was satisfied with the applicant's response.

XIV. Allergenicity and labelling

Information on this aspect is provided on p.74 of the application dossier

36. The applicant's view is that because DHC 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 5µm) 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 there is no release of the enzyme or other materials.

37. The applicant additionally highlights that no allergic reactions have been reported in workers involved in production of DHC or CH-19 Sweet extract and, although peppers have been shown to cause allergy, there are no citations relating to any effects for DHC.

***Discussion**: The Committee did not raise any concerns relating to this section of the dossier.*

CONCLUSION

The ACNFP has completed its assessment of DHC as a novel ingredient to be added to a range of foods and did not have any safety concerns relating to this ingredient. The Committee emphasised that its assessment was based purely on safety and it has not assessed or endorsed any health or taste benefits that have been suggested by the applicant. During its assessment of DHC, the Committee requested further information from the applicant on the following:

- The purpose of adding DHC to foods
- Pharmacological and nutraceutical effects of DHC or its metabolites
- Changes in blood pressure observed in the human tolerance studies
- Intakes.

After reviewing the applicant's response to these issues, the Committee did not have any outstanding safety concerns, although there was a degree of scepticism relating to the applicant's proposed reasoning for adding DHC to foods.

The ACNFP therefore concluded that DHC at the use levels proposed by the applicant will not present a health risk to consumers.

7 February 2011