Application for the Approval of Dihydrocapsiate (DHC) for Use as a Novel Food Ingredient*

NON-CONFIDENTIAL

**According to*
Regulation (EC) No 258/97 of the European Parliament
and
of the Council of 27th January 1997 concerning
novel foods and novel food ingredients

July 2010

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Executive Summary

Ajinomoto is seeking approval in Europe under Regulation (EC) No. 258/97 of the European Parliament and of the Council of 27th January 1997 concerning Novel Foods and Novel Food Ingredients, for the use of Dihydrocapsiate (DHC) as a Novel Food Ingredient. DHC occurs naturally in edible chilli peppers and therefore has a long history of consumption.

In this application Ajinomoto Co., Inc., requests approval to use DHC produced synthetically in up to 5 categories of food as food manufacturing companies have expressed interest in certain foods within these categories. The applicant is seeking approval for the use of DHC in foods that will deliver 3 mg per portion or serving. The actual DHC concentration in any food item will therefore depend upon the manufacturer's product specification for single-served products or on typical or recommended portion sizes for products presented in multi-serve packs. Using UK NDNS and EU EFSA Concise diet Database information typical and high-end (97.5th percentile or maximum) intakes were estimated for adults, children and the elderly.

The results indicated that under a conservative set of assumptions, typical intakes of the DHC Novel Food Ingredient are unlikely to exceed 0.2 to 0.4 mg/kg bw/day for an adult or 0.4 to 1.0 mg/kg bw/day for a child. Only a small percentage of the notional ADI of 10 mg/kg bw/day is utilised by these estimated figures resulting in a wide margin of safety. Higher margins of safety for acute exposure would likely exist based on the mouse oral LD_{50} value of >5,000 mg/kg bw/day. This is also borne out by the lack of any obvious adverse results during 8 days bolus dosing of DHC by capsule using healthy male volunteers treated at 12 mg/volunteer day, approximately equivalent to four servings of food, each containing 3 mg DHC, taken at a single point in time.

DHC 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 by Ajinomoto as a food supplement in the USA and Japan under the name of Capsiate Natura™.

Sourcing of large quantities of natural DHC is not sustainable because of the relatively small amounts contained in, and able to be extracted from, chilli peppers. The manufacturing process developed by Ajinomoto uses 'food-grade' equipment, storage and tight analytical controls. The product Lots produced from run to run using the commercial process produce consistently pure material.

An extensive package of toxicology conducted by the oral route has shown no evidence of toxicity or pathogenicity at dose levels of up to 1000 mg/kg bw/day over 26-weeks, and teratology and genotoxicity studies did not indicate the potential for adverse effects. Placebo controlled human studies with DHC of up to 4 weeks duration involving bolus oral administration were well tolerated with no evidence of clinically significant findings.

In conclusion, the evidence from safety studies presented in this application together with calculations of anticipated usage and use levels indicate that DHC will be safe for consumers when incorporated into specified food items as a food ingredient at concentrations designed to provide a maximum of 3 mg per serving or portion of food.

1.0 Administrative Data

1.1 Name and Address of Applicant/Manufacturer:

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2.0 General Description

2.1 The Product

The subject of the novel food ingredient application is Dihydrocapsiate (DHC).

DHC was first discovered in CH-19 Sweet, a non-pungent chilli pepper together with capsiate (Yazawa et al. 1989). Both substances are analogues of capsaicin, which have an ester bond in place of the amide bond between the vanillyl moiety and fatty acid moiety compared to capsaicin (Kobata et al., 1998). In 1999, Kobata et al., isolated a novel capsaicinoid-like substance from CH-19 Sweet, and named it nordihydrocapsiate. As a group, dihydrocapsiate (DHC), capsiate and nordihydrocapsiate are known as capsinoids. An extract from CH-19 Sweet chilli pepper which contains capsinoids, is marketed as a food supplement in America and Japan as Capsiate Natura™.

There is evidence from both human and animal studies that capsinoids (DHC, capsiate and nordihydrocapsiate) which occur naturally in edible pungent (hot) and non-pungent chilli peppers are able to enhance energy expenditure and fat oxidation. Capsinoids are chemical analogues of capsaicin which is the hot component that creates the sensation of "hotness" in chilli peppers. At high exposures the hotness can lead to a 'burning' sensation that many find pleasant in a variety of curry, goulash, and other chilli based dishes. In contrast to capsaicin, capsinoids are virtually non-pungent and have a "hot taste threshold" approximately 1000 times lower than that of capsaicin. However they still elicit some of the favourable sensory and metabolic effects experienced as a result of eating hot chilli peppers, for example it is anecdotally said that hot peppers help people in the tropics to "cool off" and are thus considered to be refreshing. Ajinomoto Co., Inc. has developed a process to produce one of the natural capsinoids, DHC, synthetically, and this substance is the object of the novel food ingredient application.

Ajinomoto proposes to use DHC as an ingredient in a wide range of food categories to allow food manufacturers flexibility in product formulation. Use levels will vary between applications but the intention is that each portion of a chosen food item containing the ingredient will deliver 3 mg of DHC. The food categories of interest to Ajinomoto include those which could be consumed by pre-school children as well as school children, adults and the elderly.

2.2 Current Status of Regulatory Approval **(Appendix 0)**

2.2.1 DHC

In the USA, DHC has been determined to be Generally Recognised as Safe (GRAS) through scientific procedures, at both 1 and 3 mg per portion of food based on standard servings[1]. The GRAS Exemption Claims dated April 21st 2008 and December 30th 2009 were reviewed by the Food and Drug Administration (FDA) who provided "no objection" Agency response letters, GRAS Notice No. GRN 000249 and GRAS Notice No. GRN 000312 on March 9, 2009 and June 6, 2010, respectively.

GRAS Notice No.GRN 000249; Appendix 0
http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/GRASListings/ucm161407.htm

GRAS Notice No.GRN 000312; Appendix 0
(The response from the FDA was provided on June 6th 2010 and the information has not yet been reported on the FDA website.)

2.2.2 CH-19 Sweet extract (Capsiate Natura™)

Concerning CH-19 Sweet extract that contains approximately 7.5% capsinoids (including approximately 20% DHC in capsinoids)

- In Europe: the Czech Republic officially recorded CH-19 Sweet extract as food supplement (2008)

[1] Reference amounts customarily consumed per eating occasion see (21 CFR 101.12)

- In Europe: the French Republic officially recorded CH-19 Sweet extract as foods (including food supplement) (2009)
- In Japan: CH-19 Sweet extract was officially recorded as a food in 2006 and has been on the market as Capsiate Natura™ since then
- In the U.S: FDA provided no objection for notification CH-19 Sweet extract as a New Dietary Ingredient in 2007 and has been on the market since then

3.0 Novel Food Categorisation

3.1 Identification of Essential Information Requirements

Approval for DHC as a novel food ingredient in Europe is sought under Regulation (EC) No 258/97[1] (as amended by Regulation (EC) No 1829/2003 and Regulation (EC) No 1882/2003) and accordingly, this submission has been prepared pursuant to the Commission Recommendation (97/618/EC)[2] of 29 July 1997.

Article 1(2) of Regulation (EC) 258/97 states that the regulation "…shall apply to the placing on the market within the Community of foods and food ingredients which have not hitherto been used for human consumption to a significant degree within the Community."

The Applicant has carefully considered the 6 categories (a to f) of novel food or novel food ingredients described by Regulation (EC) 258/97 and has confirmed with the UK Food Standards Agency as the UK Competent Authority that synthetic DHC falls within the scope of the Novel Food Regulation by virtue of it being unavailable for consumption, as such, prior to April 1997.

Section 4 of the Commission Recommendation No 97/618/EC outlines recommendations made by the Scientific Committee on Food (SCF) relating to the "Scientific Classification of Novel Foods for the Assessment of Wholesomeness" which facilitates the structured presentation of information necessary for the safety and nutritional evaluation of a given novel food or food ingredient. Of the 6 Classes identified, the Applicant considers that DHC should be allocated into Class 1: pure chemicals or simple mixtures from non-GM sources and further classified under sub-class 1.2, the source of the Novel Food has no history of food use in the Community (considering the process of production).

According to Commission Recommendation 97/618/EC, the structured schemes outlined to be followed for the assessment of a Class 1.2 novel food ingredient, are listed below. Each structured Decision Tree has been followed sequentially, using the same notation and the resultant questions are responded to point by point under sub-headings for each of the schemes.

I. Specification of the novel food

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

III. History of the organism used as the source of the novel food

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

XI. Nutritional information on the novel food

XII. Microbiological information on the novel food

XIII. Toxicological information on the novel food

[1]Regulation (EC) No 258/97 of the European Parliament and of the Council of 27th January 1997 concerning novel foods and novel food ingredients OJ L43, 14.02.1997

[2]Commission Recommendation (97/618/EC) of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients and the preparation of initial assessment reports under Regulation of (EC) No 258/97 of the European Parliament and of the Council, OJ L253,16.09.

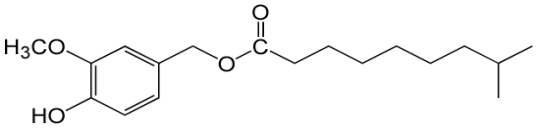
I. Specification of Dihydrocapsiate (DHC)

Based on the Commission Recommendation No 97/618 guidelines, the following questions must be answered to ensure that there is sufficient information on the specification of the novel food:

- *"Is there an appropriate specification (including species, taxon etc. for living organisms) to ensure that the novel food marketed is the same as that evaluated?"*
- *"Is the information representative of the novel food when produced on a commercial scale?"*
- *"Is appropriate analytical information available on potentially toxic inherent constituents, external contaminants and nutrients?"*

These questions have been addressed in Sections I.1 through I.3.

Ajinomoto's product for which novel food ingredient status is sought is subsequently referred to as dihydrocapsiate (DHC). It is a viscous colourless to yellow liquid which is synthesised using high purity (≤ 98% pure) starting materials.

Common or usual name:	DIHYDROCAPSIATE (DHC)
Chemical name :	(4-hydroxy-3-methoxybenzyl) 8-methylnonanoate
Synonym:	Dihydrocapsiate
CAS Number:	205687-03-2
Empirical Formula:	$C_{18}H_{28}O_4$
Structural Formula:	
Molecular Weight :	308.41
Appearance	Viscous, colourless to yellow liquid
Solubility in water	Insoluble in water
Solubility in solvents	Readily soluble in ethanol and hexane

I.1 Specification (Appendix 1)

The chemical and physical specification for commercial grade DHC has been established by Ajinomoto Co., Inc. and is given in Table 1 overleaf. Methods of analysis are also provided.

I.2 Analysis of Pilot Plant Batches for Compliance with Specification and Relationship to Commercial Grade (additionally showing microbial analysis (non-routine) and mass balance).

Seven independently manufactured lots of commercial grade DHC were produced sequentially on pilot plant scale over a 3 month period (June to August) in 2006. Each lot was analyzed to certify that the manufacturing process produces a consistent quality of product, reproducibly and within specification.

Table 1: Specification for Dihydrocapsiate (DHC)

Test items	Test Method	Acceptance criteria
Description	JSFA VII, General Notices	Viscous, colourless to yellow liquid
Identification (IR)	FCC V, Infrared Spectra	It exhibits absorption at the wave number of around 2953cm^{-1}, 2928cm^{-1}, 2855cm^{-1}, 1733cm^{-1}, 1519cm^{-1}, 1278cm^{-1}, 1241cm^{-1}, 1036cm^{-1}, 818cm^{-1} and 798cm^{-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 2.0%
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 VII, 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

A minimum mass balance of 98.14% was achieved. Over this 3 month period in 2006 it was demonstrated that the manufacturing process and final product are highly reproducible and that the process is capable of producing material that consistently meets the specification (see Table 2). The respective batches were produced in a pilot installation comprising stainless steel industrial equipment of the same type (other than scale) to be used in commercial scale production. It is thus reasonable to expect that after scale-up the production of DHC will remain within the established product specification (Table 1).

Table 2: Analysis for Compliance with Specification on 7 Commercial Grade Pilot Plant Lots and microbial analysis (non-routine)

Test item	Lot No.						
	060626	060705	060712	060720	060731	060807	060817
Description	Viscous, colourless liquid	Viscous, colourless liquid	Viscous, colourless liquid	Viscous, colourless liquid	Viscous, colourless liquid	Viscous, colourless liquid	Viscous, colourless liquid
Identification (IR)*	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms
Specific Gravity	1.030	1.030	1.028	1.028	1.024	1.026	1.025
Vanillyl alcohol (%)	0.04	0.03	0.03	0.03	<0.025	<0.025	<0.025
MNA (%)	2.0	2.0	3.3	3.4	5.8	4.3	4.8
Related substances (%)	1.10	0.86	0.74	0.69	0.81	1.39	0.75
n-Hexane (mg/kg)	<5	<5	<5	<5	<5	<5	<5
DHC Assay (%)	95.0	95.7	94.2	94.1	93.5	94.0	94.6
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
Arsenic (mg/kg)	<1	<1	<1	<1	<1	<1	<1
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
Mass Balance (%w/w)	98.14	98.59	98.27	98.22	100.11	99.69	100.15
Microbial analysis (non-routine)	ND	**total aerobic count** <3000 CFU**/g **yeast/ mould** <100 CFU/g **Coliforms** -ve	**total aerobic count** <3000 CFU**/g **yeast/ mould** <100 CFU/g **Coliforms** -ve	**total aerobic count** <3000 CFU**/g **yeast/ mould** <100 CFU/g **Coliforms** -ve	ND	ND	**total aerobic count** <3000 CFU**/g **yeast/ mould** <100 CFU/g **Coliforms** -ve

*Conforms: It exhibited absorption at the wavelength of 2953 cm^{-1}, 2928 cm^{-1}, 2855 cm^{-1}, 1733 cm^{-1}, 1519 cm^{-1}, 1278 cm^{-1}, 1241 cm^{-1}, 1036 cm^{-1}, 818 cm^{-1} and 798 cm^{-1}.

**CFU: Colony Forming Units

Quantification Limit: Residual Solvents (*n*-hexane): 5 mg/kg, Vanillyl alcohol: 0.025%, Magnesium: 0.2 mg/kg, Copper: 0.2 mg/kg, Arsenic: 0.5 mg/kg, Cadmium: 0.2 mg/kg, Lead: 0.2 mg/kg

ND: not done

I.3 Analysis for Potentially Toxic Process Impurities /External Contaminants

The presence of these substances was assessed for the starting materials and also for 7 sequentially produced Lots and the results are shown in Table 2 above. For the starting materials, see the Certificates of Analysis in Appendix 1. Considering the substances class by class:-

I.3.1 Starting Materials

As the starting materials are simple chemicals (an alcohol and a fatty acid) of very high purity (≤ 98% pure) with defined Specification, Certificates of Analysis and associated Material Safety Data Sheets (MSDS) purchased from approved major chemical suppliers, and held under hygienic practices there is negligible opportunity for contact with external contaminants. Similarly because of the defined Specification and quality checks on the starting materials via Certificates of Analysis to show compliance, there is very little likelihood that they could contain undetected *'inherent'* toxicants. Vanillyl alcohol, is listed in the United States of America, Food and Drug Administration (FDA) EAFUS ("Everything" Added to food in the United States), Database, which is maintained by the Center for Food and Drug Safety and Applied Nutrition (CFSAN) under a programme known as the Priority-based Assessment of Food Additives (PAFA) [1].

Vanillyl alcohol

Synonyms: vanillyl alcohol, o-vanillyl alcohol, 4-hydroxy-3-methoxybenzenemethanol, 4-(hydroxymethyl)-2-methoxyphenol, vanillic alcohol, vanillin alcohol
Molecular weight: 154.16
Molecular formula: $HOC_6H_3(OCH_3)CH_2OH$
CAS No: 498-00-0
EINECS No: 207-852-4
Purity: Not less than 98.8%
Appearance: white to tan crystalline powder
Odour: Mild, sweet, balsamic, vanilla-like
Melting point: 112.00 to 115.00 °C. @ 760.00 mm Hg
Boiling Point: 312.00 to 313.00 °C. @ 760.00 mm Hg
Flash point: 289.00 °F. TCC (142.78 °C)
Toxicology/safety: May act as a skin, eye or respiratory irritant.
Use: food flavouring

8-Methylnonanoic acid
Synonyms: 8-methyl nonanoic acid, Isocapric acid
Molecular Weight: 172.26
Molecular formula: C10H20O2
CAS No: 5963-14-4
EINECS: Unlisted
Purity: Not less than 98.0%
Appearance: colourless oil
Odour: waxy
Melting point: 20 to 25 ºC at 760.00 mmHg
Boiling Point: 118 to 120 ºC at 2 mmHg
Flash point: N/A
Toxicology/safety: May cause irritation. May be harmful by inhalation, ingestion or skin absorption.
Use: Food

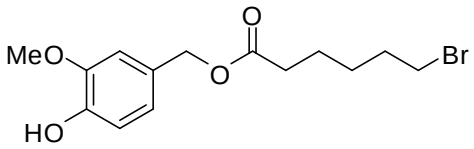
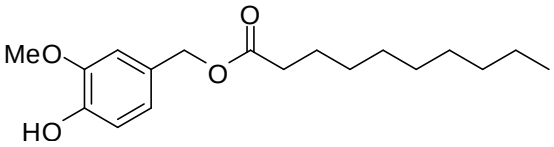
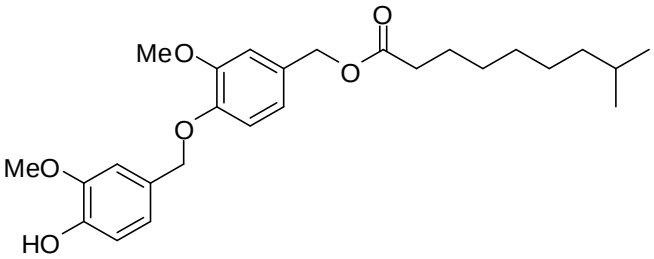
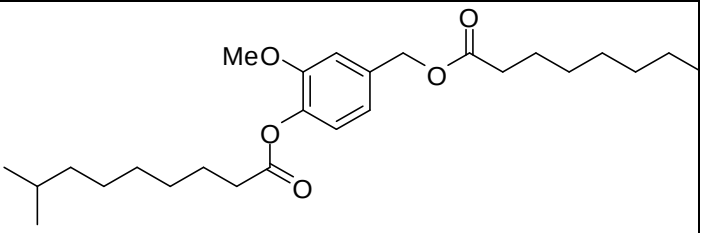
[1]PAFA contains administrative, chemical and toxicological information on over 2000 substances directly added to food, including substances regulated by the U.S. Food and Drug Administration (FDA) as direct, "secondary" direct, and color additives, and Generally Recognized As Safe (GRAS) and prior-sanctioned substances. In addition, the database contains only administrative and chemical information on less than 1000 such substances. The more than 3000 total substances together comprise an inventory often referred to as *"Everything" Added to Food in the United States* (EAFUS).

I.3.2 Reaction Impurities

As shown in Table 2, 'related substances' are routinely analysed in each production lot. In the 7 Lots reported in Table 2 the levels of related substances comprised between 0.69% and 1.39%.

Analysis carried out to identify and quantify these substances showed 4 main peaks (which accounted for 77% to 91% of total other related substances). These were identified and are presented in Figure 1.

Figure 1: Chemical Structures Identified as 'Related Substances' in Commercial Grade Pilot Plant Lots

Name	Structure
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	

I.3.3 Solvents

The only solvent employed in the synthesis of DHC is *n*-hexane which is employed for quenching the esterification reaction as well as for extraction of the DHC following filtration, (see Figure 2, Section II). It has been specified with a maximum upper limit in the final product of 5 mg/kg. *n*-Hexane is a solvent which is flammable and has the potential for neurotoxicity following chronic exposure. Human exposure by inhalation to 5000 ppm has been shown to cause side effects. However the residues in DHC are reproducibly below 5 ppm, providing a 1000-fold margin of exposure MOE (ie comparing inhalation to oral exposure). In consequence the presence of very low levels of *n*-hexane that could occur in DHC are considered unlikely to pose a risk for consumers when it is taken into account that the ingredient will be significantly diluted by the food matrix to which it is added. Use is governed by Council Directive 88/344/EEC on the approximation of the laws of the Member States on extraction solvents used in the production of foodstuffs and food ingredients,13 June 1988.

I.3.4 Metals

Of the heavy metals analysed, namely arsenic, cadmium, and lead, these were below the limits of detection in all Lots analysed. In the case of lead Commission Directive 2008/60/EC of 17th June 2008

laying down specific purity criteria concerning sweeteners for use in food stuffs, Commission Directive 2008/84/EC of 27th August 2008 laying down specific purity criteria on food additives other than colours and sweeteners, and, Commission Directive 2008 laying down specific purity criteria concerning colours for use in foodstuffs, all provide maximum levels between 0.5 and 20 mg/kg for lead as an impurity in numerous food additives. As lead was found to be present in DHC below the level of detection (LOD) at <0.2 mg/kg, lead is not considered to represent a significant hazard. For arsenic, specific purity criteria concerning sweeteners, colours and other food additives are laid down in 3 Commission Directives, 2008/60/EC, 2008/84/EC and 2008/128/EC. All Directives provide maximum limits (MLs) of 3 mg/kg for arsenic as an impurity in several food additives. On the basis was not found to exceed 1 mg/kg in DHC, arsenic is not considered to represent a potential hazard. With regard to cadmium Regulation (EC) no. 629/2008 refers to MLs of cadmium in food supplements of between 1 and 3 mg/kg. As cadmium levels in each of the 7 Lots of DHC were below the limit of detection of 0.2 mg/kg, these levels can again be seen to lie well below the MLs for food supplements. In consequence the low levels of heavy metals analysed in DHC are not considered to pose a risk to the consumer of DHC incorporated as a novel food ingredient into a range of foodstuffs.

Copper is regulated in the EU at 1 mg/kg in food products whereas magnesium has an upper limit of 250 mg/day/person/day which is approximately equivalent to 4.2 mg/kg bw/day for a 60 kg person. In the case of both cations the specification limit is set at 1 mg/kg in the DHC Commercial grade product. As the maximum DHC content per food serving is just 3 mg, the maximum cation content that could enter food is negligible. Moreover the 3 mg DHC/food serving, and any residual cations, will further be diluted by orders of magnitude by the bulk of the foodstuff into which it is added. It is thus considered that the levels of copper and magnesium, which are at or below the limits of detection in the DHC Lots analysed, will pose no risk to to the consumer of DHC incorporated as a novel food ingredient into a range of foodstuffs.

I.3.5 Microbial Contaminants (See Section XII)

The presence of a range of potential microbial contaminants has been assessed by an independent certified laboratory using Lots 060705, 060712, 060720 and 060817 of DHC. No coliforms were detected in any of the batches and the counts for the different groups of microorganisms were within the range for similar dry ingredients used as food grade materials (See also Section XII).

I.3.6 Possibility of Protein Content (See Section II.2.1)

The only potential source of protein entering the chemical based production process would be from the enzyme lipase used for the esterification reaction. The enzyme, Novozym® 435FG, (Novozymes, Denmark) is supplied by the manufacturer in an immobilised form embedded in a solid inert methacrylate granulate carrier (average size approximately 500 µm, range 150 – 900 µm) which means that the enzyme cannot partition into the *n*- hexane fraction which contains the extracted DHC. However even in the 'worst case' that granulate "fines" entered the hexane layer, the particles would be trapped during the filtration process which uses filters of 5 µm porosity, and would thus be separated from the DHC product. Furthermore the Novozymes lipase employed, (Novozym® 435FG), has no record of associated allergy or enzyme release since the registration to the best of the knowledge of the manufacturer.

In conclusion, with the Specification of DHC described in Table 1, the toxicological characteristics for each of the substances described above, at the levels typically occurring in simulated production runs on a pilot plant scale, and the planned commercial manufacturing, handling and storage (at -20 ºC) of DHC, to GMP and hygienic standards, there are no qualitative or quantitative grounds to consider that the DHC product will be unwholesome when incorporated into foodstuffs at the anticipated inclusion levels (Section IX).

II. Effect of the Production Process Applied to the Novel Food Ingredient

Based on the Commission Recommendation No 97/618 guidelines, the following questions must be addressed to ensure sufficient information exists on the production process for the novel food ingredient:

- *"Does the novel food undergo a production process?"*
- *"Is there a history of use of the production process for the food?" If no …*
- *"Does the process result in a significant change in the composition or structure of the NF compared to its traditional counterpart?"*

These questions have been addressed collectively in Sections II.1 through II.3

II.1 Description of the Production Process

The manufacturing process was developed in order to generate a reproducible, high quality, within specification DHC product that could be made in quantity on demand. In contrast, the extraction of DHC from chilli peppers, while feasible, is environmentally challenging due to a very low extraction rate. Additionally it is subject to the seasonality of chilli growing and compositional variation in capsinoid content due to biotic and abiotic factors.

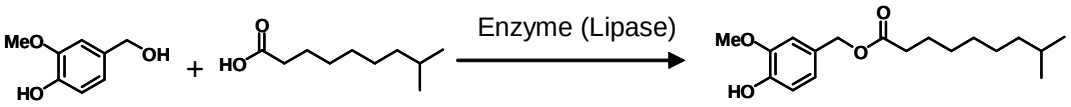
A synthetic production process not only meets the above criteria but is widely used for many other food ingredients such as enzymes and vitamins. The use of a synthetic approach is thus a well established route and utilises well proven food ingredient manufacturing technology.

The production process is summarized in Figure 2 on the following page. Please refer to Section I Table 2 for compositional data on typical production batches.

The manufacture of DHC begins with the esterification of vanillyl alcohol (V-OH) and 8-methylnonanoic acid (MNA) using an immobilized food grade lipase preparation purchased from Novozymes, Denmark.

Figure 2: Process Flow Chart for Dihydrocapsiate Manufacture

The esterification is catalyzed by a lipase produced by Novozymes, Denmark (Novozym® 435 FG) and approved by Denmark as a processing aid.



V-OH MNA Dihydrocapsiate

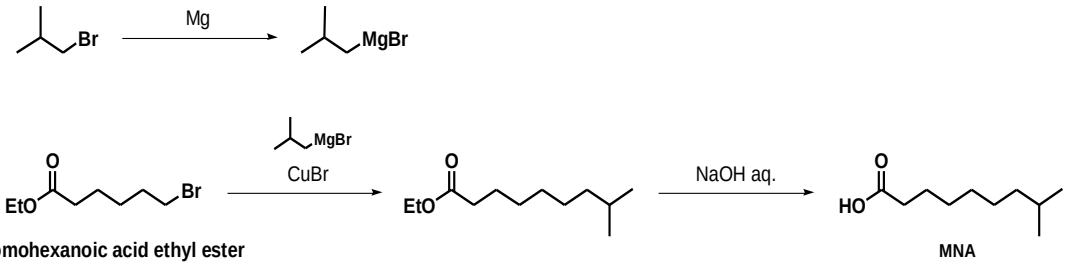
II.2 History of Use of the Production Process

The pilot plant production process for DHC is a simple batch process which is capable of manufacturing between 10 and 20 kg lots reproducibly to specification on each production 'run'. Each run takes approximately 5 days including reaction, extraction, drying and transfer to storage.

II.2.1 Raw Materials and Processing Aids

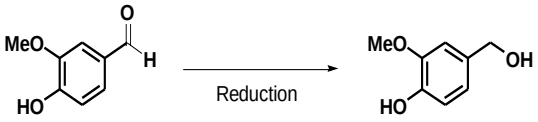
The starting, raw materials vanillyl alcohol and 8-methylnonanoic acid are simple substances, an alcohol and a fatty acid, that have been found to be present as intermediary metabolites in chilli peppers and thus have been consumed by man over centuries. Vanillyl alcohol is sold as a flavouring for food and 8-methylnonanoic acid is also used in the food industry.

MNA is prepared from isobutyl bromide and 6-bromohexanoic acid ethyl ester through a Grignard coupling reaction and de-protection process to give the carboxylic acid.



6-Bromohexanoic acid ethyl ester **MNA**

V-OH is prepared from vanillin by reduction. Following reduction, evaporation is conducted.



Vanillin **V-OH**

Lipase, produced by Novozymes Denmark, (Novozym® 435 FG)[1] and recently (April 2010) renamed Lipozyme 435, is approved by the Danish Veterinary and Food Administration under MAFF as a processing aid for interesterification of oils and fats in food applications in Denmark and hence throughout the EU (approval reference No. 2006-20-5406-00106) and see Appendix 1 for the Product Data Sheet, Material Safety Data Sheet and Danish Health Certificate. The product complies with the recommended purity specifications for food-grade enzymes given by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Food Chemical Codex (FCC). The enzyme is immobilised on an inert methacrylate carrier and studies have been made by Novozymes on the potential leaching of enzyme or components from the carrier material under normal use. The results have shown that Novozym® 435 FG is a robust product under normal handling and application with no release of protein or other materials. Abrasion, grinding or other abnormal physical treatments would be required to cause the carrier granules to be degraded and no such physical action occurs during the production of DHC. As a further reassurance the Ajinomoto process uses 3 post-reaction filtration steps during the course of production, using 5 micron filters. As the Novozym® 435 FG granulate product is approximately 500 microns in size the filtration steps are considered to virtually eliminate any granulate and hence enzyme from the final product.

II.2.2 Description of the Manufacturing Process

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

[1] Novozym® 435 FG Declared activity 1000 PLU/g

II.2.3 Potential Impurities Resulting from the Production Process

Impurities have been characterized in the final novel food ingredient. Two types are considered:

- Starting material residues
- Reaction products

II.2.3.1 Starting material residues
Starting substances or solvent (*n*-hexane) used for DHC extraction are potential residues in the final food ingredient. However, as indicated in Section I (Specification of the novel ingredient), numerous analytical controls are made on the final product, DHC, to ensure that the levels have no implications for the health of consumers.

Results on seven batches produced over 3 months in 2006 are shown in Section I, Table 2.

II.2.3.2 Reaction products - other related substances
As shown in Figure 1 (Section I, Specification) other related substances were identified in the 7 Lots analyzed. The levels of these substances comprised between 0.69% and 1.39%.

An analysis was carried out to identify and quantify these substances in the 7 production batches analysed see (Table 2). Several peaks (which accounted for 77% to 91% of total other related substances) were identified. Of these, vanillyl dihydrocapsiate was the largest individual impurity with a concentration of 0.73%

II.3 Structural Identity and Stability of dihydrocapsiate (DHC)

II.3.1 Does the Process Result in a Significant Change in the Composition or Structure of the Novel Food Ingredient Compared to its Traditional Counterpart?

The Pilot Plant process produces DHC with an identical chemical structure to the natural DHC that can be extracted, and is consumed, from hot chilli peppers.

II.3.2 Stability

The stability of DHC has been studied in different packaging and under a variety of environmental conditions. Typically the material is stored at -20 ºC in 1 Kg amounts in aluminium pouches held in sealed containers.

Table 3 below provides a summary of stability studies on DHC. The test parameters included *inter alia* purity, identification, description of appearance and other related substances

These results show that DHC is stable for a period of at least 2 years at either 5 ºC or -20 ºC. Therefore, the shelf life of the product was determined to be 12 months (as a minimum).

Table 3: Stability of Dihydrocapsiate over 24 months vs. Storage Temperature (Lot No: 070813)

Test items		Initial	6 months	12 months	24 months
Storage conditions: -20ºC ± 5ºC					
Description		Viscous colourless liquid	Viscous colourless liquid	Viscous colourless liquid	Viscous colourless liquid
Identification (IR Spectrophotometry)		Conforms	NT	NT	NT
Specific Gravity		1.03	NT	NT	NT
Related Substances	Vanillyl alcohol (%)	0.12	0.12	0.11	0.11
	Total of other related substance (%)	0.59	0.59	0.57	0.66
Side chain fatty acid (MNA)	Content (%)	3.9	3.9	3.9	4.0
Dihydrocapsiate	**Content (%)**	**95.7**	**95.7**	**94.6**	**95.1**
Storage Condition: 5ºC ± 3ºC					
Test items		Initial	6 months	12 months	24 months
Description		Viscous, colourless liquid	Viscous, colourless liquid	Viscous, colourless liquid	Viscous, colourless liquid
Identification (IR Spectrophotometry)		Conforms	NT	NT	NT
Specific Gravity		1.03	NT	NT	NT
Related Substances	Vanillyl alcohol (%)	0.11	0.34	0.37	0.58
	Total of other related substance (%)	0.71	0.62	0.63	0.70
Side chain fatty acid (MNA)	Content (%)	3.9	4.1	4.2	4.5
Dihydrocapsiate	**Content (%)**	**95.4**	**95.2**	**94.7**	**94.3**

NT = Not tested

III. History of Peppers as the Original Source of the Novel Food Ingredient (including background human exposure to the Novel Food Ingredient and its source).

Based on the Commission Recommendation No 97/618 guidelines, the first question must be addressed to ensure sufficient information pertaining to the history of the original source organism. The further 3 questions are also answered to provide an understanding of the history of food use of the source of the Novel Food Ingredient as Section X information is not required for a Sub-Class 1.2 novel ingredient :

- *"Is the novel food obtained from a biological source (i.e., a plant, animal or microorganism)?"*
- *"Has the organism used as the source of the novel food been derived using genetic modification?"*
- *"Is the source organism characterised?"*
- *"Is there information to show that the source organism and/or foods obtained from it are not detrimental to human health?"*

These questions have been addressed in Sections III.1 through III.4, respectively

III.1 Natural Occurrence of DHC in the Diet from Chilli Peppers (Appendix 2)

DHC occurs naturally in the human diet as it is naturally present in chilli peppers. It belongs to the chemical family called capsinoids (typically DHC, nordihydrocapsiate and capsiate) which are present in most chilli peppers, which come from the large genus Capsicum. Capsicum terminology is confusing. Pepper, chili, chile, chilli, aji, paprika, and Capsicum are used interchangeably for a range of plants in the genus Capsicum. The word "chili" or 'chilli' is a variation of "chil" derived from the Nahuatl (Aztec) dialect which referred to plants now known as Capsicum, whereas "aji" is a variation of "axi" from the extinct Arawak dialect of the Caribbean, (Domenici 1983).

The capsinoid family, containing DHC, is one of the natural constituents of the 5 domesticated chilli peppers (*Capsicum annuum*, *Capsicum frutescens*, *Capsicum chinense*, *Capsicum baccatum*, Capsicum pubescens) and sweet peppers like Paprika (*Capsicum annuum var annuum* L.) (IBPGR 1983). It is estimated that the typical daily intake of DHC ranges from approximately 0.01 to 0.06 mg/kg bw/day, (Sub-section III.4 below).

The taxonomy of peppers is as follows:-

Division : Magnoliophyta

Class : Magnoliopsida

Subclass : Asteridae

Order: Solanales

Family: Solanaceae

Genus: Capsicum

More than 20 different domesticated species of Capsicum have been identified by Walsh and Hoot (2001). In addition the genus Capsicum also includes approximately 22 wild species. (Bosland 1994).

The most common species of chilli peppers are:

- ***Capsicum annuum***, which includes many common varieties such as bell peppers, paprika, cayenne, jalapeños, piperoni or chiltepin

Different cultivars are available which include:

Capsicum annuum var. *acuminatum*; *Capsicum annuum* var. *angulosum* Synonym: *Capsicum annuum* var. *grossum* (Mill); *Capsicum annuum* var. *aviculare* Synonym: *Capsicum annuum* var. *glabriusculum*
Capsicum annuum var. *anomalum*. Synonym: *Turbocapsicum anomalum* (Makino); *Capsicum annuum* var. *baccatum* Synonym: *Capsicum baccatum* var. *baccatum* (Kuntze)
Capsicum annuum var. *cerasiforme*; *Capsicum annuum* var. *conoide*; *Capsicum annuum* var. *conicum*, (GFW Meyer - Cone pepper); *Capsicum annuum* var. *cordiforme* (Edwall); *Capsicum annuum* var. *cuneatum* (Paul); *Capsicum annuum* var. *fasciculatum*
Capsicum annuum var. *glabriusculum* Synonym: *Capsicum annuum* var. *aviculare*; *Capsicum annuum* var. *minimum* (Heiser); *Capsicum hispidum* var. *glabriusculum* (Dunal)
Capsicum annuum var. *grossum* (Sendt); *Capsicum annuum* var. *leucocarpum* (Kuntze); *Capsicum annuum* var. *longum* (Bailey)
Capsicum annuum var. *luteum* (Lam); *Capsicum annuum* var. *lycopersiciforme* (Auquier); *Capsicum annuum* var. *minimum* Synonym: *Capsicum annuum* var. *glabriusculum*; Capsicum *annuum* var. *minus* Synonym: *Capsicum annuum* var. *annuum*; *Capsicum annuum* var. *microcarpum*; *Capsicum annuum* var. *parvo-acuminatum* Synonym: *Capsicum annuum* var. *acuminatum*
Capsicum annuum var. *pyramidale* (Mill); *Capsicum annuum* var. *violaceum* (Humboldt, Bonpland and Kunth)

- ***Capsicum frutescens***, which includes the tabasco peppers
- ***Capsicum chinense***, which includes the hottest peppers such as the naga, habanero and Scotch bonnet
- ***Capsicum pubescens***, which includes the South American rocoto peppers
- ***Capsicum baccatum***, which includes the South American aji peppers

Figure 3: Different Cultivars of Peppers (see **http://en.wikipedia.org/wiki/Capsicum**)

III.1.1 Other Dietary Sources of DHC

As DHC is present in the capsinoids contained in most peppers, it can also be found in food products made from these plants both fresh or dried eg when used whole or as spices, flavourings, colourings, powders, extracts, hot sauces (eg Tabasco), barbecue sauces, ketchup, cheese, snack foods, dips, and meals such as chilli con carne, salads and sausages:

- The spices *Capsicum* (plant source: *Capsicum frutescens* L. or *Capsicum annuum* L.) and paprika (plant source: *Capsicum annuum* L.) are among the spices and other natural seasonings and flavourings that are generally recognized as safe (GRAS) in USA for their intended use in food (21CFR182.10; 582.10).

- *Capsicum* and paprika are also listed among the essential oils, oleoresins (solvent-free), and natural extractives (including distillates) that are GRAS in USA for their intended use in food (21CFR182.20; 582.20).
- Paprika (ground dried pod of mild capsicum [*Capsicum annuum* L.]) may be used safely for the colouring of foods, generally, in amounts that are consistent with good manufacturing practice (21 CFR:73.340)
- Paprika extract and oleoresin of Paprika have been evaluated by the JECFA (1970, 2008) for their uses as food colourings and flavourings.

Exposure to capsinoids also occurs through food supplements ie Capsiate Natura™ (CH-19 Sweet extract) sold in America and Japan (Section 2.2.2).

III.2 Non-GM Status of the Source

We are not aware of the production of genetically modified chilli peppers.

III.3 History of Pepper Consumption and Estimates of Background DHC Intake

Peppers represent a wide number of varieties. This taxon includes both sweet cultivars eaten mainly as vegetables and hot ones, often used as a spice. These plants belong to the Solanaceae family (Conforti et al. 2007).

Capsicum has been known since the beginning of civilization in the Western Hemisphere. It has been a part of the human diet since about 7500 BC (MacNeish 1964). It was the ancient Aztec ancestors of the native peoples who took the wild chili piquin and selected for the many various types known today. Heiser (1976) states that apparently between 5200 and 3400 BC, the Native Americans were growing chilli plants. This places chillies among the oldest cultivated crops of the Americas. As opposed to most domesticated crops, the wild ancestral chillies are not looked upon as worthless or inferior by farming people who cultivate their domestic decedents. The wild Capsicum annuum var. aviculare is harvested and sold in the marketplace along side the larger-fruited domesticated chillies. Capsicum was domesticated at least five times by prehistoric peoples in different p arts of South and Middle America.

Red peppers were consumed for more than 6000 years in Mexico (Walsh and Hoot 2001). They were firstly mentioned by the botanist and doctor, Nicolas Monardes, who travelled with Christopher Columbus in 1494.

They were then introduced in Europe and identified in Italy in 1526, in Germany in 1542, in Hungary in 1569, in Great-Britain and in France at the early 17th century.

In Europe, capsicums were consumed as spice in place of pepper or ginger mainly because of their low cost and easy cultivation.

Subsequently, red peppers were introduced all over the world and are considered today to be the most consumed spice.

Sweet peppers are widely consumed and cultivated in Europe and mainly in countries around the Mediterranean sea such as Spain, France and Italy and in Central and Eastern Europe (i.e., Hungary, Bulgaria). They are also widely grown and consumed in North Africa, California and New Mexico as well as in the tropics and Caribbean. Peppers and pepper containing products enter U.K. market from all over the world with a very large variety and diversity available on most supermarket shelves.

Some examples of food uses are given by Wikipedia: see http://en.wikipedia.org/wiki/Capsicum

Capsicum fruits and peppers can be eaten raw or cooked. Those used in cooking are generally varieties of *the C. annuum* and *C. frutescens* species. They are also frequently used both chopped and raw in salads, or cooked in stir-fries or other mixed dishes, such as curries, con carne, goulash etc . They can be sliced into strips and fried, roasted whole or in pieces, or chopped and incorporated into salsas or other sauces. Peppers may also be preserved by drying, pickling or freezing. Dried peppers may be reconstituted whole, or processed into flakes or powders. Pickled or marinated peppers are frequently added to sandwiches or salads. Extracts can be made and incorporated into hot sauces.

In 2005, a poll of 2,000 people revealed the capsicum pepper to be Britain's 4th favourite culinary vegetable.

In Bulgaria, peppers are very popular, too. They can be eaten in salads, like Shopska Salata; fried and then covered with a dip of tomato paste, onions, garlic, and parsley; or stuffed with a variety of products - like minced meat and rice, beans, or cottage cheese and eggs. Peppers are also the main ingredient in the traditional tomato and pepper dip - lyutenitsa. They are in the base of different kinds of pickled vegetables dishes - turshiya.

III.3.1 Natural Occurence of Capsinoids and DHC in Capsicums

Several analytical studies report levels of capsinoids (which contain DHC) and capsaicinoids in capsicum fruits (Table 4). Yazawa et al (2004) reported levels of total capsinoids in 18 cultivars ranging from not detected (detection limit not given) to 1,818 mg/kg (DW) in the selected cultivar 'CH-19 sweet'. Levels in non- selected cultivars ranged up to 1106 mg/kg (DW). Whilst certain sweet peppers contained no capsinoid or capsaicinoid, some 'hot' cultivars contained small amounts of capsinoid and large amounts of capsaicinoid. It was considered that the capsinoid biosynthetic pathway is closely related with the capsaicinoid pathway. However, there does not appear to be a quantitative relationship between the two compounds (Figure 4). Although there is no statistically significant correlation, the line fit indicates a negative relationship.

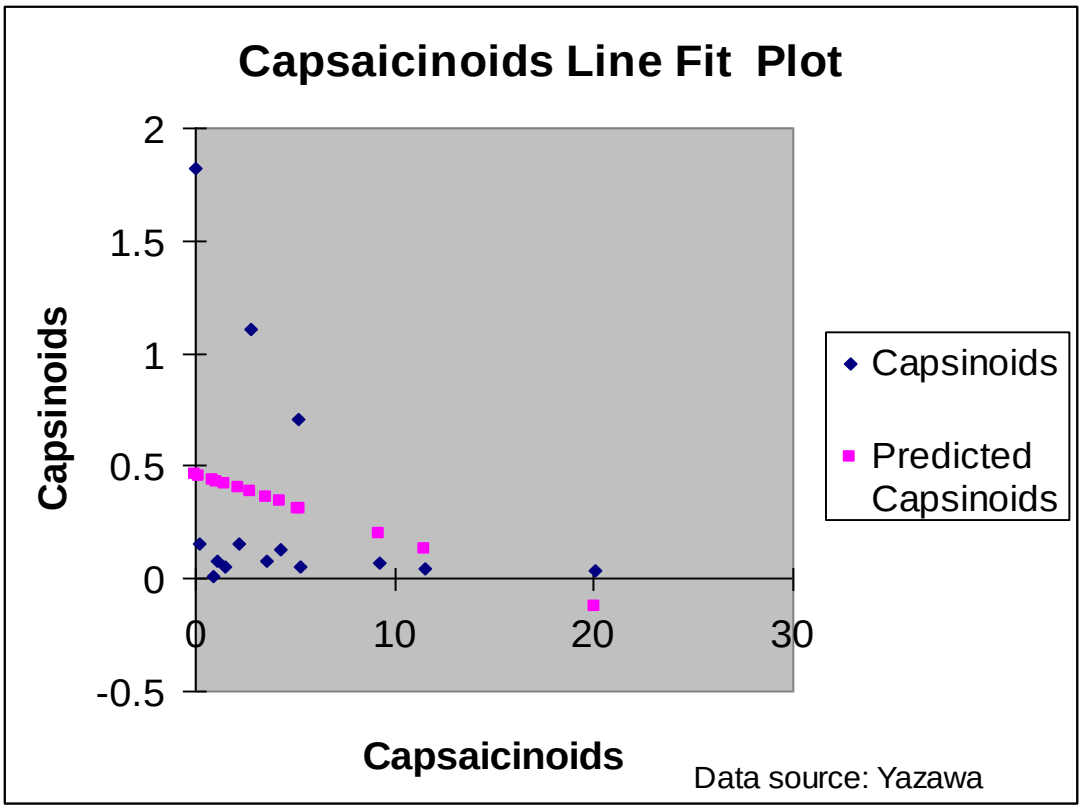


Figure 4 Relationship between Capsinoids and Capsaicinoids in some Capsicum Fruits.

Singh *et al* initially reported the results of the capsinoids (E-capsiate (which is the capsiate optical isomer present in nature) and DHC) analysis of capsicum fruits harvested from USDA Agricultural Research Services cultivars in 2007. The same cultivars were re-analysed and new cultivars analysed in 2009. From the reported results is possible to investigate the relationship between total capsinoids and DHC (Figure 5) or E-capsiate and DHC in these fruits (Figure 6). This shows a reasonable correlation (R=0.875) and indicates that DHC concentrations can be predicted from either E-capsiate or total capsiate levels.

The correlation for total capsinoids and DHC gives a negative value on the x-axis and since it must therefore pass through zero and the equation becomes:

DHC = Total capsinoids*0.2

For E-capsiate the equation is:

DHC = E-capsiate*0.24+0.026

These equations can be used to predict DHC values where results have not been reported (Table 4).

Table 4: Reported levels of Capsinoids and Calculated Levels of DHC in Cultivars of Capsicum

	Sample	Capsinoids (mg/g DW)	*Capsinoids (mg/g FW)*	E-capsiate (mg/kg FW)	dihydrocapsiate (mg/kgFW)	*DHC (Calculated)*
Yazawa et al 2004	CH-19 Sweet	1818	*181.8*			*36*
	Nikko	159	*15.9*			*3*
	Tekanotsume	155	*15.5*			*3*
	Tsumura	40	*4*			*1*
	Yokaku	51	*5.1*			*1*
	Goshiki	131	*13.1*			*3*
	Enomi	73	*7.3*			*1*
	Cayenne long slim	79	*7.9*			*2*
	Hungarian yellow wax	9	*0.9*			*0*
	Habanero	37	*3.7*			*1*
	Af-8	52	*5.2*			*1*
	Yatsuhusa	82	*8.2*			*2*
	Puchi marble	12	*1.2*			*0*
	Aroma-Af3	1106	*110.6*			*22*
	Sy-2	710	*71*			*14*
	California wonder	< ?				*0*
	Murasaki	< ?				*0*
	Shishitoh	< ?				*0*
Singh et al 2007	PB26			410	96	*98*
	PE56			274	81	*66*
	NP30			185	55	*44*
	NP42			185	20	*44*
	PE86			184	37	*44*
	PE31			176	24	*42*
	NP37			152	39	*37*
	441680			144	13	*35*
	PE66			136	39	*33*
	PB35			121	51	*29*
Singh et al 2009	PB 26			369.1	86.9	*89*
	PE 56			246.9	73.3	*59*
	PE 84			189.7	nd	*46*
	NP 30			166.7	49.8	*40*
	NP 42			166.3	18	*40*
	PE 86			166.2	33.7	*40*
	PE 31			158.7	21.4	*38*
	NP 37			137.5	34.8	*33*
	GRIF 9302			132.3	nd	*32*
	PE 66			122.6	35.5	*29*
	PE 67			116.4	nd	*28*
	NP 28			110.3	nd	*26*
	PB 35			109.6	45.5	*26*
	PB 31			105.7	nd	*25*
	PE 12			83.8	nd	*20*
	PE 50			81.4	nd	*20*
	PE 75			80	nd	*19*
	PI 438644			79.9	nd	*19*
	PB 22			75.7	nd	*18*
	PE 16			71.5	nd	*17*
	PE 23			64.7	nd	*16*
	PE 59			60	nd	*14*
	PE 80			57.8	nd	*14*
	NP 13			55.4	nd	*13*
	PE 82			53.1	nd	*13*
	PE 33			46	nd	*11*
	PB 38			45.8	nd	*11*
	P21			43.3	nd	*10*
	NP 47			40.6	nd	*10*
	PI 224424			40.4	nd	*10*
	NP 1			38.3	nd	*9*
	PE 37			35.5	nd	*9*
	PI 438535			32.4	nd	*8*
	NP 32			31.5	nd	*8*
	PB 93			30.7	nd	*7*
	PB 18			29.9	nd	*7*
	NP 4			22.2	nd	*5*
	PE 06			20.8	nd	*5*
	PE 05			20.6	nd	*5*
	NP 23			19.5	nd	*5*
	PE 79			19	nd	*5*
	NP 5			17.8	nd	*4*
	PE 85			17.7	nd	*4*
	PE 54			16.7	nd	*4*
	PI 439476			14.7	nd	*4*
	PB 25			13.5	nd	*3*
	NP 35			12.9	nd	*3*
	PE 61			11.7	nd	*3*
	PE 7			11.5	nd	*3*

E-capsiate ; the optical isomer present in nature

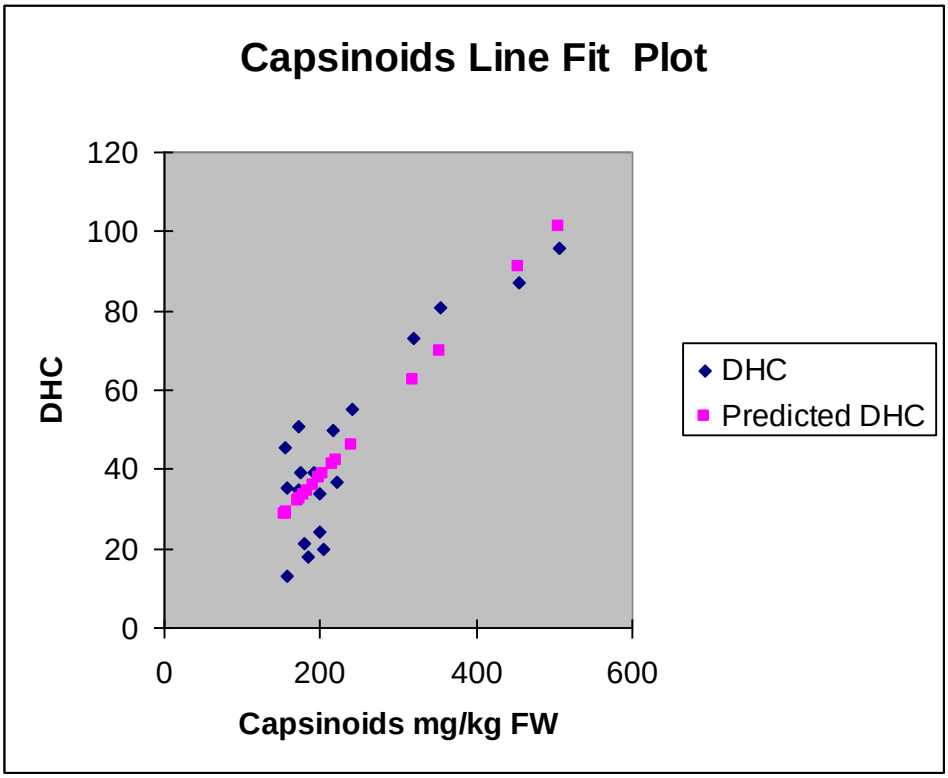


Figure 5 Relationship between Capsinoids and Dihydrocapsiate in some Capsicum Fruits.

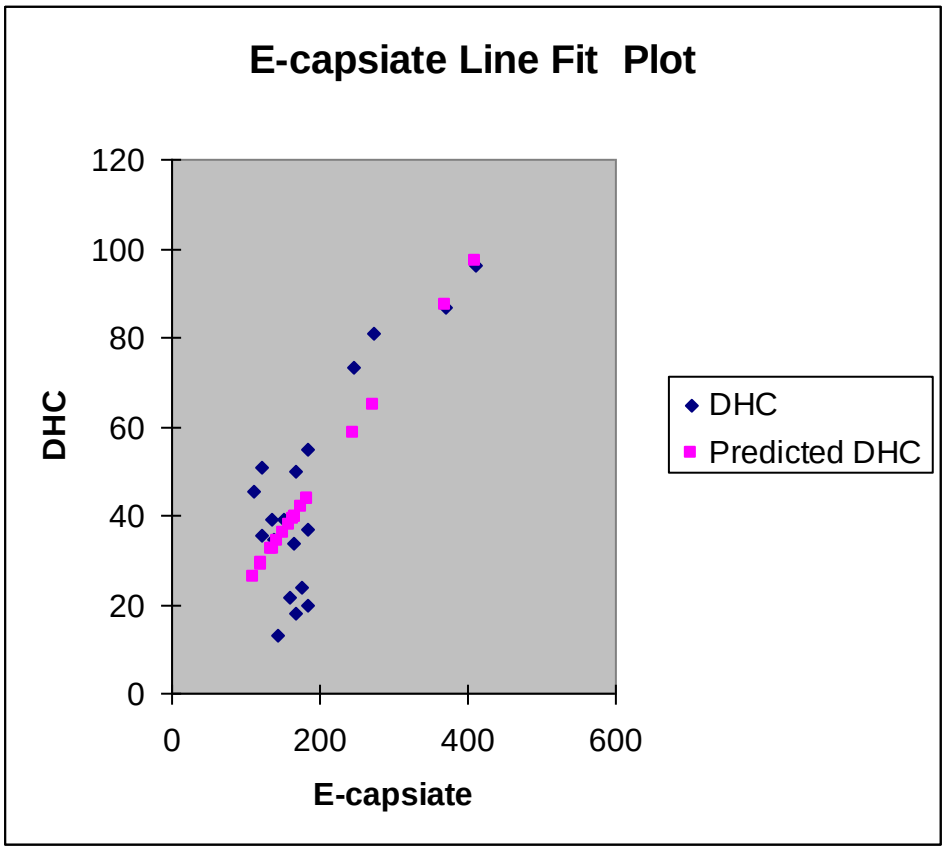


Figure 6 Relationship between E-capsiate* and Dihydrocapsiate in some Capsicum Fruits.

*the optical isomer present in nature

The combined data from Yazawa et al and Singh et al can be used to describe the potential distribution of DHC in capsicums in the human food chain (Table 4). There is no evidence that hot and sweet capsicums contain significantly different levels of DHC and so all data have been combined into one distribution (Figure 7).

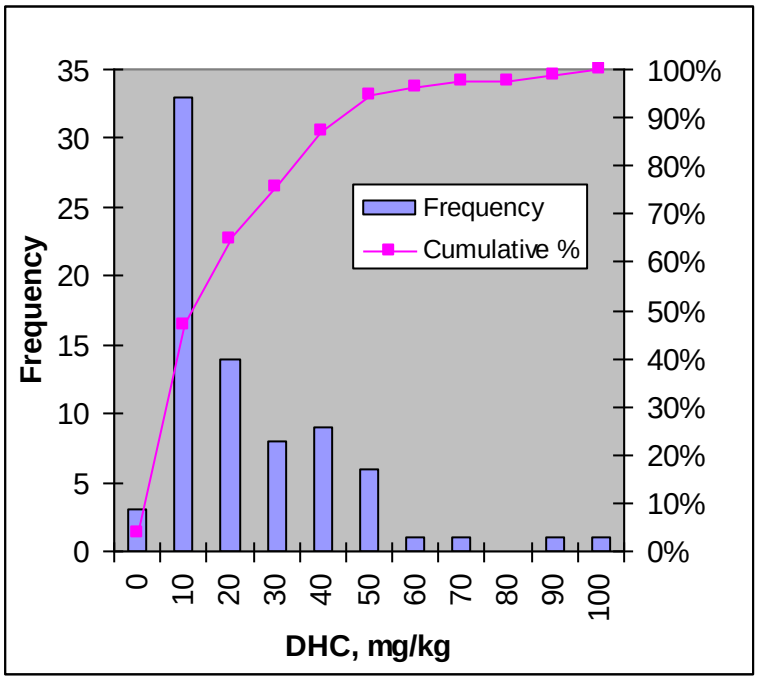


Figure 7 Distribution of Calculated DHC Levels in Analysed Samples of Capsicums.

The average concentration of DHC in capsicums was 19 mg/kg and the 90th, 95th and 97.5th percentiles 43, 48 and 68 mg/kg respectively. It is unclear what value should be used in intake estimates since the levels are very variable and highly skewed. Furthermore, concentrations within the same cultivar show considerable variability (Singh et al 2009). It seems reasonable to assume that for the typical consumer over the longer term exposure will be to average levels. However, this does not exclude the possibility that a consumer always consuming a particular cultivar of capsicum could have a significantly higher exposure.

III.3.2 Natural Intakes of Capsinoids Including DHC from Capsicums

The UN Food and Agriculture Organisation Statistical Service (FAOSTAT) food balance sheets provide a complete balance for all agricultural commodities (including food crops) split-up into the following sections:

> The first section deals with domestic supply of the various commodities such as production, imports, stock changes and exports.
>
> The second section deals with domestic utilization, which includes the use of commodities for feed, seed, processing, waste, other uses, and food.
>
> The third section provides per-capita values for the supply of all commodities (in kg per person per year). This section additionally provides corresponding values for the calories, protein and fat content.

The most recent currently available Food Balance Sheet (FBS) data for capsicums come from 2004 and 2005. The data relate to the domestic supply of fresh and dried peppers so can be assumed to include sweet peppers, chillies and dried products such as paprika, spices, etc. Dried pepper consumption can be converted to wet (fresh) weight using a notional factor of 10. Data are available for 157 global countries (Table 5).

Table 5 FAO Food Balance Sheet data for Capsicums and Estimated Dihydrocapsiate Intakes

	Fresh 2004	Fresh 2005	Dry 2004	Dry 2005	Total (Fresh) Ave 2004/5	DHC intake mg/day		DHC intake mg/kg bw/day	
Countries	g/day	g/day	g/day	g/day	g/day	**Average**	**High**	**Average**	**High**
Bosnia and Herzegovina	30.82	36.18	20.85	19.59	235.70	4.5	16.0	0.07	0.27
Hungary	14.73	15.14	15.59	17.29	179.34	3.4	12.2	0.06	0.20
Jamaica	4.94	5.54	12.26	13.32	133.14	2.5	9.1	0.04	0.15
Macedonia,The Fmr Yug Rp	127.03	128.94	0.14	0.09	129.14	2.5	8.8	0.04	0.15
Tunisia	66.88	65.33	0.8	0.78	74.01	1.4	5.0	0.02	0.08
Ghana	33.8	34.15	3.56	3.88	71.18	1.4	4.8	0.02	0.08
Romania	27.67	30.03	4.05	4.21	70.15	1.3	4.8	0.02	0.08
Turkey	55.16	54.57	0.64	0.6	61.07	1.2	4.2	0.02	0.07
Malaysia	0.13	0.13	5.74	6.32	60.43	1.1	4.1	0.02	0.07
Dominica	0	0	6.46	5.4	59.30	1.1	4.0	0.02	0.07
Benin	8.81	8.73	4.57	4.55	54.37	1.0	3.7	0.02	0.06
Mexico	35.01	34.27	1.71	1.76	51.99	1.0	3.5	0.02	0.06
Bulgaria	49.46	48.6	0.16	0.07	50.18	1.0	3.4	0.02	0.06
Sri Lanka	5.35	5.37	3.59	3.73	41.96	0.8	2.9	0.01	0.05
Ethiopia	2.58	2.73	3.95	3.9	41.91	0.8	2.8	0.01	0.05
Albania	38.56	39.52	0.01	0	39.09	0.7	2.7	0.01	0.04
Myanmar	0	0	3.68	3.81	37.45	0.7	2.5	0.01	0.04
Spain	33.82	33.81	0.32	0.27	36.77	0.7	2.5	0.01	0.04
Slovenia	14.39	14.88	2.17	2.23	36.64	0.7	2.5	0.01	0.04
Egypt	16.53	15.36	1.71	1.74	33.20	0.6	2.3	0.01	0.04
Bangladesh	0	0.01	2.84	2.82	28.31	0.5	1.9	0.01	0.03
Czech Republic	11.56	11.66	1.65	1.68	28.26	0.5	1.9	0.01	0.03
Morocco	15.39	16.49	1.11	1.13	27.14	0.5	1.8	0.01	0.03
China	23.77	25	0.25	0.24	26.84	0.5	1.8	0.01	0.03
Thailand	0.27	0.32	2.52	2.68	26.30	0.5	1.8	0.01	0.03
Viet Nam	0	0	2.5	2.5	25.00	0.5	1.7	0.01	0.03
India	0.12	0.12	2.47	2.5	24.97	0.5	1.7	0.01	0.03
Korea, Republic of	17.91	18.78	0.62	0.67	24.80	0.5	1.7	0.01	0.03
Algeria	16.36	17.27	0.77	0.79	24.62	0.5	1.7	0.01	0.03
Israel	25.78	23.16	0	0	24.47	0.5	1.7	0.01	0.03
Croatia	21.15	18.62	0.4	0.4	23.89	0.5	1.6	0.01	0.03

Table 5 continued.

Countries	Fresh 2004 g/day	Fresh 2005 g/day	Dry 2004 g/day	Dry 2005 g/day	Total (Fresh) Ave 2004/5 g/day	DHC intake mg/day Average	DHC intake mg/day High	DHC intake mg/kg bw/day Average	DHC intake mg/kg bw/day High
Greece	22.01	21.04	0.21	0.15	23.33	0.4	1.6	0.01	0.03
Côte d'Ivoire	3.13	3.18	1.96	1.92	22.56	0.4	1.5	0.01	0.03
Slovakia	16.78	17.41	0.46	0.5	21.90	0.4	1.5	0.01	0.02
Kuwait	14.51	14.39	0.69	0.73	21.55	0.4	1.5	0.01	0.02
Nigeria	12.35	12.24	0.86	0.84	20.80	0.4	1.4	0.01	0.02
Cambodia	0	0	1.97	1.97	19.70	0.4	1.3	0.01	0.02
Cuba	18.17	20.33	0	0	19.25	0.4	1.3	0.01	0.02
Nepal	1.25	1.42	1.75	1.8	19.09	0.4	1.3	0.01	0.02
United States of America	11.27	11.46	0.75	0.74	18.82	0.4	1.3	0.01	0.02
Austria	10.72	11.23	0.75	0.73	18.38	0.3	1.2	0.01	0.02
Grenada	0	0	1.95	1.67	18.10	0.3	1.2	0.01	0.02
Maldives	2.7	2.68	1.57	1.47	17.89	0.3	1.2	0.01	0.02
Pakistan	0	0	1.65	1.75	17.00	0.3	1.2	0.01	0.02
Netherlands	10.86	10.93	0.59	0.59	16.80	0.3	1.1	0.01	0.02
Barbados	12.9	13.35	0.34	0.37	16.68	0.3	1.1	0.01	0.02
Italy	16.44	16.68	0.01	0.01	16.66	0.3	1.1	0.01	0.02
Brunei Darussalam	2.09	2.25	1.96	0.91	16.52	0.3	1.1	0.01	0.02
Congo, Dem Republic of	0	0	1.56	1.48	15.20	0.3	1.0	0.00	0.02
Moldova, Republic of	13.6	13.72	0.02	0.03	13.91	0.3	0.9	0.00	0.02
Zimbabwe	0	0	1.4	1.38	13.90	0.3	0.9	0.00	0.02
Libyan Arab Jamahiriya	6.32	5.83	0.56	0.8	12.88	0.2	0.9	0.00	0.01
Denmark	8.64	8.97	0.36	0.37	12.46	0.2	0.8	0.00	0.01
Sweden	8.24	8.39	0.37	0.38	12.07	0.2	0.8	0.00	0.01
Germany	8.3	8.42	0.37	0.37	12.06	0.2	0.8	0.00	0.01
Sierra Leone	0.03	0.01	1.2	1.16	11.82	0.2	0.8	0.00	0.01
Uruguay	9.68	9.15	0.21	0.26	11.77	0.2	0.8	0.00	0.01
Indonesia	10.03	10.34	0.06	0.05	10.74	0.2	0.7	0.00	0.01
Argentina	8.29	8.48	0.23	0.23	10.69	0.2	0.7	0.00	0.01
Kazakhstan	9.6	10.37	0.06	0.06	10.59	0.2	0.7	0.00	0.01
Togo	0	0	1.06	1.01	10.35	0.2	0.7	0.00	0.01
Djibouti	0	0.62	0.92	1.05	10.16	0.2	0.7	0.00	0.01
Switzerland	7	7.09	0.3	0.29	10.00	0.2	0.7	0.00	0.01
Canada	6.46	5.92	0.38	0.38	9.99	0.2	0.7	0.00	0.01

Table 5 continued.	Fresh 2004	Fresh 2005	Dry 2004	Dry 2005	Total (Fresh) Ave 2004/5	DHC intake mg/day		DHC intake mg/kg bw/day	
Countries	g/day	g/day	g/day	g/day	g/day	**Average**	**High**	**Average**	**High**
Mauritius	2.49	2.64	0.71	0.74	9.82	0.2	0.7	0.00	0.01
Saint Vincent/Grenadines	9.57	9.44	0.02	0.02	9.71	0.2	0.7	0.00	0.01
Senegal	0	0	0.88	1.06	9.70	0.2	0.7	0.00	0.01
Guyana	9.46	8.57	0.07	0.03	9.52	0.2	0.6	0.00	0.01
Australia	6.43	6.82	0.24	0.24	9.03	0.2	0.6	0.00	0.01
Iceland	8.35	9.59	0	0.01	9.02	0.2	0.6	0.00	0.01
Venezuela,Bolivar Rep of	8.5	9.07	0.01	0.01	8.89	0.2	0.6	0.00	0.01
Cameroon	1.1	1.14	0.8	0.71	8.67	0.2	0.6	0.00	0.01
Chile	8.68	8.38	0	0	8.53	0.2	0.6	0.00	0.01
United Kingdom	5.29	5.58	0.26	0.27	8.09	0.2	0.5	0.00	0.01
Norway	5.48	5.56	0.23	0.23	7.82	0.1	0.5	0.00	0.01
Belgium	4.03	4.02	0.36	0.38	7.73	0.1	0.5	0.00	0.01
Ukraine	7.06	7.15	0.04	0.05	7.56	0.1	0.5	0.00	0.01
Dominican Republic	7.1	7.08	0.02	0.02	7.29	0.1	0.5	0.00	0.01
Cyprus	6.34	6.33	0.08	0.08	7.14	0.1	0.5	0.00	0.01
Ireland	5.35	5.58	0.15	0.16	7.02	0.1	0.5	0.00	0.01
El Salvador	6.09	6.99	0.04	0.01	6.79	0.1	0.5	0.00	0.01
Finland	4.18	4.3	0.23	0.23	6.54	0.1	0.4	0.00	0.01
Korea, Dem People's Rep	6.15	6.34	0.02	0.03	6.50	0.1	0.4	0.00	0.01
Estonia	3.46	3.45	0.29	0.28	6.31	0.1	0.4	0.00	0.01
Sudan	0.45	0.49	0.58	0.58	6.27	0.1	0.4	0.00	0.01
Lebanon	4.91	4.95	0.1	0.13	6.08	0.1	0.4	0.00	0.01
Japan	3.42	3.42	0.25	0.27	6.02	0.1	0.4	0.00	0.01
Niger	4.21	4.87	0.11	0.1	5.59	0.1	0.4	0.00	0.01
France	4.39	4.55	0.11	0.11	5.57	0.1	0.4	0.00	0.01
Iran, Islamic Rep of	4.39	4.66	0.09	0.09	5.43	0.1	0.4	0.00	0.01
Saudi Arabia	1.1	0.97	0.36	0.46	5.14	0.1	0.3	0.00	0.01
Syrian Arab Republic	4.77	4.6	0.03	0.01	4.89	0.1	0.3	0.00	0.01
Tanzania, United Rep of	0.17	0.21	0.45	0.45	4.69	0.1	0.3	0.00	0.01
Paraguay	4.57	4.3	0.01	0.01	4.54	0.1	0.3	0.00	0.01
Madagascar	0.01	0.01	0.46	0.43	4.46	0.1	0.3	0.00	0.01
Luxembourg	3.16	2.81	0.14	0.13	4.34	0.1	0.3	0.00	0.00
South Africa	0	0	0.41	0.43	4.20	0.1	0.3	0.00	0.00

Table 5 continued.

Countries	**Fresh 2004** g/day	**Fresh 2005** g/day	**Dry 2004** g/day	**Dry 2005** g/day	**Total (Fresh) Ave 2004/5** g/day	**DHC intake mg/day** Average	High	**DHC intake mg/kg bw/day** Average	High
Belize	4.02	4.38	0	0	4.20	0.1	0.3	0.00	0.00
Fiji Islands	2.76	2.94	0.13	0.12	4.10	0.1	0.3	0.00	0.00
Latvia	3.53	3.76	0.04	0.04	4.05	0.1	0.3	0.00	0.00
Kenya	0.4	0.37	0.37	0.36	4.04	0.1	0.3	0.00	0.00
Seychelles	0.82	1.32	0.23	0.36	4.02	0.1	0.3	0.00	0.00
Jordan	3.82	4.06	0	0.01	3.99	0.1	0.3	0.00	0.00
Portugal	1.68	1.79	0.2	0.2	3.74	0.1	0.3	0.00	0.00
Uganda	0	0	0.37	0.36	3.65	0.1	0.2	0.00	0.00
New Zealand	1.37	1.24	0.19	0.16	3.06	0.1	0.2	0.00	0.00
Guatemala	2.66	3.01	0	0.03	2.99	0.1	0.2	0.00	0.00
Malawi	0	0	0.27	0.28	2.75	0.1	0.2	0.00	0.00
Poland	0.77	0.78	0.19	0.2	2.73	0.1	0.2	0.00	0.00
Lithuania	1.41	1.41	0.09	0.08	2.26	0.0	0.2	0.00	0.00
Yemen	1.92	1.97	0	0	1.95	0.0	0.1	0.00	0.00
Colombia	2	1.84	0	0	1.92	0.0	0.1	0.00	0.00
Azerbaijan, Republic of	1.71	1.89	0	0	1.80	0.0	0.1	0.00	0.00
Panama	1.87	1.7	0	0	1.79	0.0	0.1	0.00	0.00
Russian Federation	0.92	1.11	0.06	0.07	1.67	0.0	0.1	0.00	0.00
Bolivia	1.63	1.6	0	0	1.62	0.0	0.1	0.00	0.00
Burkina Faso	1.43	1.39	0	0	1.41	0.0	0.1	0.00	0.00
Malta	0.85	1.04	0.04	0.05	1.40	0.0	0.1	0.00	0.00
Ecuador	1.08	1.43	0	0	1.26	0.0	0.1	0.00	0.00
Uzbekistan	0.23	0.16	0.11	0.1	1.25	0.0	0.1	0.00	0.00
Honduras	1	1.09	0.01	0	1.10	0.0	0.1	0.00	0.00
Botswana	0.88	0.79	0.01	0	0.89	0.0	0.1	0.00	0.00
Philippines	0.58	0.56	0.02	0.02	0.77	0.0	0.1	0.00	0.00
Zambia	0	0	0.07	0.07	0.70	0.0	0.0	0.00	0.00
Central African Republic	0	0	0.06	0.07	0.65	0.0	0.0	0.00	0.00
Kyrgyzstan	0.06	0	0.05	0.07	0.63	0.0	0.0	0.00	0.00
Georgia	0.09	0.11	0.05	0.05	0.60	0.0	0.0	0.00	0.00
Belarus	0.18	0.22	0.03	0.03	0.50	0.0	0.0	0.00	0.00

Table 5 continued.	Fresh 2004	Fresh 2005	Dry 2004	Dry 2005	Total (Fresh) Ave 2004/5	DHC intake mg/day		DHC intake mg/kg bw/day	
Countries	g/day	g/day	g/day	g/day	g/day	**Average**	**High**	**Average**	**High**
Namibia	0.03	0.01	0.05	0.04	0.47	0.0	0.0	0.00	0.00
Suriname	0	0	0.04	0.05	0.45	0.0	0.0	0.00	0.00
Armenia	0	0	0.04	0.03	0.35	0.0	0.0	0.00	0.00
Trinidad and Tobago	0.01	0	0.02	0.03	0.26	0.0	0.0	0.00	0.00
Peru	0.01	0	0.02	0.02	0.21	0.0	0.0	0.00	0.00
Liberia	0.11	0.17	0	0.01	0.19	0.0	0.0	0.00	0.00
Costa Rica	0.25	0.06	0	0	0.16	0.0	0.0	0.00	0.00
Swaziland	0.02	0.07	0.01	0.01	0.15	0.0	0.0	0.00	0.00
Samoa	0.09	0.09	0	0	0.09	0.0	0.0	0.00	0.00
Mongolia	0.02	0.01	0.01	0	0.07	0.0	0.0	0.00	0.00
Angola	0	0	0.01	0	0.05	0.0	0.0	0.00	0.00
Gambia	0	0	0.01	0	0.05	0.0	0.0	0.00	0.00
Kiribati	0.03	0.06	0	0	0.05	0.0	0.0	0.00	0.00
Vanuatu	0.01	0.05	0	0	0.03	0.0	0.0	0.00	0.00
Gabon	0.01	0	0	0	0.01	0.0	0.0	0.00	0.00
Brazil	0	0	0	0	0.00	0.0	0.0	0.00	0.00
Burundi	0	0	0	0	0.00	0.0	0.0	0.00	0.00
Chad	0	0	0	0	0.00	0.0	0.0	0.00	0.00
Comoros	0	0	0	0	0.00	0.0	0.0	0.00	0.00
Congo, Republic of	0	0	0	0	0.00	0.0	0.0	0.00	0.00
Guinea	0	0	0	0	0.00	0.0	0.0	0.00	0.00
Guinea-Bissau	0	0	0	0	0.00	0.0	0.0	0.00	0.00
Haiti	0	0	0	0	0.00	0.0	0.0	0.00	0.00
Mozambique	0	0	0	0	0.00	0.0	0.0	0.00	0.00
Nicaragua	0	0	0	0	0.00	0.0	0.0	0.00	0.00
Rwanda	0	0	0	0	0.00	0.0	0.0	0.00	0.00
Sao Tome and Principe	0	0	0	0	0.00	0.0	0.0	0.00	0.00
Solomon Islands	0	0	0	0	0.00	0.0	0.0	0.00	0.00
Turkmenistan	0	0	0	0	0.00	0.0	0.0	0.00	0.00

The countries with the highest consumption of fresh capsicums were the former Yugoslavian Republic of Macedonia, Tunisia, Turkey and Bulgaria. The countries with the highest consumption of dried capsicums were Bosnia and Herzegovina, Hungary, Jamaica, Dominica and Malaysia. The countries with the highest overall consumption were Bosnia and Herzegovina, Hungary, Jamaica, Macedonia, Tunisia and Ghana.

DHC intakes have been calculated for average consumers assuming average capsicum consumption and average levels of DHC (19 mg/kg) and for high level consumers using the 97.5th DHC level (68 mg/kg).). It was not possible to use 97.5th percentiles for consumption in this case because the food balance sheet (FBS) database does not include upper percentile consumption data. Average intakes ranged from 0 – 4.5 mg/day (0 – 0.07 mg/kg bw/day for 60 kg adult) and high level intakes up to 16 mg/day (0.27 mg/kg bw/day for 60 kg adult). For an individual who consumes more than average amounts of capsicums or with a lower body weight intakes could be significantly higher.

Potential intakes of DHC by UK consumers have been investigated using a probabilistic model. Consumption of peppers has been estimated using NDNS data for adults described in Section IX.1. Because hot chillies and sweet peppers are often used as ingredients in other foods is has been assumed that chilli dishes contain 0.05% capsicums and that other dishes containing peppers as an ingredient contain 0.2% capsicums. Recipes were included in the assessment where 'chillies', 'curry' or 'peppers' were mentioned in the food description. Food descriptions and ingredient correction factors are provided in Appendix 2.1. Consumption of peppers by UK consumers is summarised in Table 6. The results are compatible with FAO/FBS estimates of *per capita* consumption of peppers by UK consumers (8.09 g/day) and are consistent with the data for pepper consumption provided in the UK Pesticides Safety Directorate chronic consumption model (Pesticides Safety Directorate download).

Table 6 Consumption of Capsicums by UK Consumers

			Consumption, g/day				Consumption, g/kg bw/day			
	N	**%**	**Mean**	**P90**	**P95**	**P97.5**	**Mean**	**P90**	**P95**	**P97.5**
Chillies (incl dishes)	24	1%	2	6	6	9	0.04	0.07	0.09	0.20
Dried chilli, etc.	30	2%	1	3	4	4	0.02	0.04	0.05	0.07
Curry recipes	576	34%	3	6	7	10	0.04	0.08	0.11	0.14
Sweet peppers (Incl dishes)	516	30%	11	25	29	34	0.14	0.32	0.41	0.49
All above	899	53%	8	20	27	31	0.11	0.27	0.35	0.43

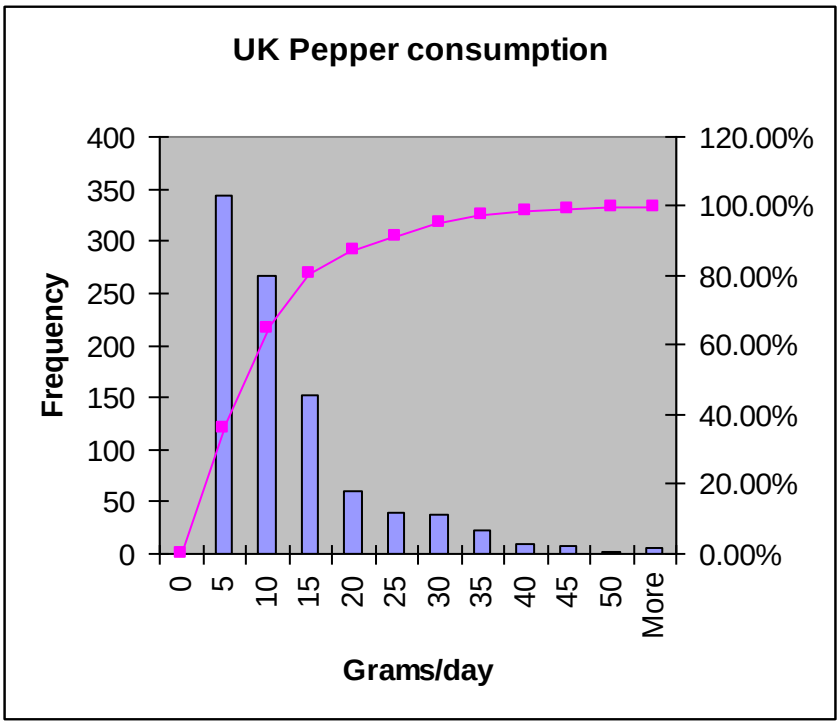


Figure 8 Distribution of Consumption of Capsicums by UK adults (Consumers only)

The distribution of consumption is, like the levels of DHC in capsicums, highly skewed towards lower values (Figure 9).

Intakes of DHC can be estimated by combining the distribution of DHC values (Figure 5) with the distribution of possible pepper consumption values (Figure 8). In the probabilistic model, each of the distributions is re-sampled many times to create a distribution of possible intake values (Figure 9).

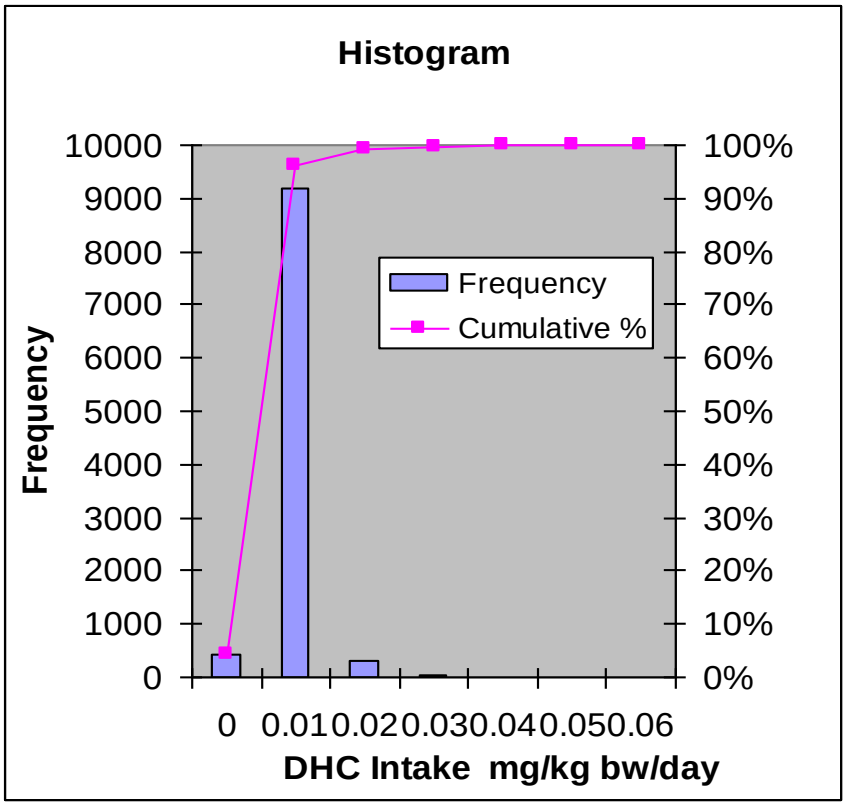


Figure 9 Probabilistic Distribution of DHC Intakes (Consumers only).

The results indicate that whilst the majority of DHC intakes are likely to be very low (less than 0.01 mg/kg bw/day) a small proportion may be as high as 0.06 mg/kg bw/day (Table 7). In the longer term it is likely that these differences would disappear as each individual was exposed to a wider variety of cultivars.

Table 7 Potential intake of DHC by UK adults from natural occurrence

Mean	0.002	
P90	0.005	
P95	0.009	
P97.5	0.012	
P99	0.018	
P99.9	0.037	
Maximum	0.059	mg/kg bw/day

III.3.3 Conclusion on Previous Human Exposure to Dihydrocapsiate (DHC)

DHC appears to occur in many cultivars of capsicums but at varying and unpredictable levels, from the limited number of analysed samples. The distribution of natural occurrence levels appears to be log-normally distributed with a mean or median value around 20 mg/kg with higher levels approaching 100 mg/kg. There does not appear to be any particular association with 'hot' chilli cultivars containing higher levels of capsaicinoids. In fact the cultivar selected for breeding to produce high levels of DHC is a 'sweet' variety. It seems likely that in the longer term individuals will be exposed to fruits with a variety of DHC concentrations. However, it is possible that if an individual always chooses a particular cultivar, and that cultivar happens to contain higher than normal levels of DHC, then he/she could have a significantly higher exposure than the average person.

Natural intakes of DHC will depend upon the consumption of hot and sweet capsicums as well as levels of DHC. Data on average per capita consumption of capsicums published by FAO indicate that intakes could be as low as zero 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 Eastern European countries where capsicums are consumed in larger quantities.

A probabilistic model was used to investigate potential intakes for more typical European consumers in the UK. This showed that consumption of capsicums was also probably log-normal with an average (for consumers) or 8 g/day rising to about 30 g/day for high level consumers. This resulted in very low intakes (less than 0.01 mg/kg bw/day) for the majority of consumers with a small proportion with intakes as high as 0.06 mg/kg bw/day.

IX Anticipated Intake/Extent of Use of the Novel Food Ingredient

Based on the Commission Recommendation No 97/618 guidelines, the following questions must be addressed to ensure sufficient information pertaining to the anticipated intake and extent of use of the novel food:

- *"Is there information on the anticipated uses of the novel food based on its properties?"*
- *"Is there information to show anticipated intakes for groups predicted to be at risk?"*
- *"Will introduction of the novel food be restricted geographically?"*
- *"Will the novel food replace other foods in the diet?"*

These questions have been addressed in Sections IX.1 through IX.4.

IX.1 Anticipated Use and Use-levels in Food

Dihydrocapsiate (DHC) is intended to be available for use in a wide range of food categories in order to allow food manufacturers flexibility in product formulation. The Applicant has had discussions with food manufacturers which have enabled them to identify certain foods in five categories – baked goods, beverages, confectionery, cereals and desserts and other miscellaneous foods where the potential to use DHC as a novel food ingredient exists.

The Applicant, Ajinomoto Co., Inc., plans to produce the food ingredient for use by third party food manufacturers but will not itself manufacture foods containing DHC. In consequence Ajinomoto Co., Inc., does not have information about specific product formulations and in particular, package volumes for foods in which DHC might be presented. As a consequence, the applicant is seeking approval for use of DHC that will deliver 3mg per portion or serving. This is similar in approach to previous novel food applications where an applicant has provided information about the use level required to achieve a particular level of intake, based on notional portion sizes.

Actual use levels will vary between applications so that each portion of a given food product will deliver 3 mg of DHC. The actual DHC concentration in any food item will therefore depend upon that manufacturer's product specification for single serve products or on typical or recommended portion sizes for products presented in multi-serve packs. An individual's total daily intake of DHC will depend on how many servings of food containing 3 mg of DHC he or she consumes. In order to develop reliable projections of anticipated intakes to evaluate the dietary and nutritional significance of the novel food, it has been necessary to estimate use levels for DHC in the potential range of food categories. In the absence of a standard list of portion sizes, in making these estimates it has been assumed that use levels have been adjusted so that an average serving for an UK adult consumer will deliver 3 mg of DHC.

The UK NDNS survey of adults (Henderson et al, 2002) has been analysed to determine the average quantity of food consumed at every eating occasion for each of the potential food categories. These values are presented in Table 8. For foods such as dilutable drinks or where the product is presented as a powder, such as instant coffee, the quantity reported represents the total amount consumed, including water for dilution. The concentrations in the food (as consumed) necessary to achieve an intake of 3 mg per serving have been calculated from the serving size data and are also provided in Table 8. These concentrations will be used to model potential intakes of UK individuals with normal dietary food consumption patterns.

IX.2 Anticipated Intakes of Dihydrocapsiate (EU)

Because it is not yet known which of the potential food categories will be selected by food manufacturers to present DHC to the market, a hypothetical worst-case scenario has been adopted which assumes that *all* of the potential food applications contain sufficient DHC to provide 3 mg DHC from an average serving of food. This maintains a conservative approach since if a larger portion size was used (e.g. 97.5th percentile), then the DHC concentration required to provide 3mg per serving would be lower. In reality it is unlikely that the market would support such a diverse range of food applications and so the resulting estimates of intake will over-estimate true potential exposures. A detailed assessment has been provided for UK consumers based on individual food consumption data. Intake estimates have also been generated using European Food Safety Authority (EFSA) Concise Diets data for 15 EU countries and Norway to provide a general view of potential intakes across the EU (EFSA, 2008).

Table 8: Average UK Adult Serving Size (g) and Potential Uses and Use-levels of Dihydrocapsiate

Group	Application	Average Portion Size (g)*	Conc. to provide 3 mg (mg/kg)	
Baked goods				
	Cereal bars	33	90.1	Bars - Breakfast and meal replacement bars
	Biscuits & cookies	33	90.5	Cookies – low fat and non-fat
	Crackers	32	94.7	Low-fat and non-fat crackers
	Rice-based snacks	25	119.0	Snack foods – popped-low-calorie – low fat and rice cakes
Beverages				
	Dilutable drinks	250	12.0	Beverage concentrates
	Carbonated drinks	267	11.2	Beverages soft drinks
	Energy drinks	368	8.1	Energy drinks
	Fruit juice-based drinks	241	12.5	Fruit juice drinks, squashes
	Drink mixes	209	14.3	Fruit-aides, drinks, and powders
	Coffee-based drinks	212	14.1	Liquid coffee; instant coffee
	Meal replacement drinks	320	9.4	Meal replacement beverages
	Flavoured water - still	285	10.5	Non-carbonated water – low calorie
	Tea-based drinks	208	14.4	Tea, liquid, powdered, herbal
	Vegetable juice	169	17.8	Vegetable juice
Confectionery				
	Sugar-free gum	3	1133.9	Chewing gun – sugarless
	Hard candy	11	269.8	Hard candy, (including mints)
	Chocolate confectionery	40	74.8	Chocolate confectionery
Cereals and desserts				
	Frozen ices	86	34.8	Frozen desserts – dairy
	Frozen dairy desserts	82	36.5	Frozen desserts - ice pops and fruit bars
	Pudding mixes	140	21.4	Gelatin/Puddings – low-calorie
	Yogurt (not fruit)	117	25.7	Yogurt – Chocolate, Vanilla, Plain
	Yogurt (fruit-flavoured)	142	21.2	Yogurt – Fruit
	Instant oatmeal cereal	123	24.4	Oatmeal – instant
	Other cereals	71	42.2	Ready to eat cereals, breakfast cereals, cereal desserts

Table 8: Continued

Other foods

Ready-to eat frozen meals	276	10.9	Ready to eat meals – frozen (non-meat, -chicken, -fish/seafood)
Soup	280	10.7	Ready to eat meals – soup
Whitener/creamer	8	382.8	Creamer
Replacement meal	265	11.3	Nutritional meal
Vegetable protein	61	49.5	Protein based meat alternative; soy-based products
Salad dressing	18	164.3	Salad dressings
Sweeteners	1	2049.6	Sweeteners – low-calorie

* Average quantity consumed per serving occasion, based on NDNS adults' data

IX.2.1 Individual Intakes based on UK National Dietary and Nutrition Survey Data

The UK National Dietary and Nutrition Survey (NDNS) programme of adults, children (pre-school and school age) and the elderly was designed to provide a comprehensive cross-sectional picture of the dietary habits and nutritional status of the population of Great Britain (Table 9). In each survey the sample covered individuals living in private households. The method of data collection was by weighed diaries over 7 or 4 days.

Table 9 . The UK National Diet and Nutrition Survey (NDNS) Data

Survey	Year(s)	Age range	Participants*
Children Aged 1½ to 4½. (Gregory et al, 1995)	1992-1993	1½ to 4½	1,675
Young People Aged 4 to 18 Year (Gregory, 2000)	1997	4 to 18	1,686
Adults aged 19 to 64 years (Henderson et al, 2002)	2000 - 2001	19 to 64	2,251
People Aged 65 Years and Over. (Finch 1998)	1994-1995	65 +	1,632

* Not all participants provided a complete set of data, in particular some body-weights were missing.

The NDNS data comprise records of the amounts of more than 7,000 different food items consumed by each individual in the survey on every eating occasion. For the DHC analyses, each of the NDNS food codes was matched to a usage application for DHC or set aside as not used (Appendix 2.1).

Computer-based statistical software was used to calculate the consumption of each food by each individual in the survey, averaged over the duration of the survey, in a distributional model. The average and 90th, 95th and 97.5th percentiles are calculated from the distributions to represent typical and high level consumption. DHC intakes from each food group are estimated by applying the DHC concentration provided in Table 8 to each NDNS food description and then calculating each individual's intake, averaged over the duration of the survey. Each individual's total intake is estimated by summing intakes from the food groups. The average and 90th, 95th and 97.5th percentile DHC intakes are then calculated from the distribution for each food group and for all foods combined. Data for intake from individual foods are presented as intakes for only those individuals who reported consumption of that food during the survey.

Because the portions sizes used to estimate the quantity of food required to deliver 3 mg of DHC represent a source of uncertainty in the approach an alternative method has also been employed where it is assumed that on each and every occasion that an individual consumes any of the foods that might contain DHC, they receive a 3 mg ingredient – regardless of the portion size. In this assessment an intake of 3 mg DHC is assigned to each eating occasion so that the total intake is a reflection of the number of occasions on which any of the foods was consumed.

Estimates of intake of DHC for UK consumers can be expressed on a per kg bodyweight basis by dividing by each individual's bodyweight reported in the survey. Because children have higher energy demands they frequently have higher intakes of food constituents than adults when expressed on a bodyweight basis. Although it is not expected that DHC products will be especially selected for consumption by children, potential intakes have been provided in order to represent a maximum potential intake scenario.

Detailed descriptions of potential intakes by UK consumers in four age groups (adult, pre-school children, and elderly) are provided in Appendix 2.2. Maximum potential intakes based on use in all proposed food groups are summarised for UK consumers in Table 10 and 11. The results are consistent with the results obtained for UK adults using the EFSA Concise Diet database whilst confirming the conservatism inherent in that approach. Children's intakes are generally lower than adults when expressed in mg/day, probably reflecting smaller portions sizes. However, their intakes are higher when expressed on a mg/kg bodyweight basis, reflecting their lower bodyweights.

Table 10 Potential Intakes* based on Levels Required to Achieve 3 mg in Average Adult Portion

	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

When intakes are reassessed on the basis that a 3 mg DHC is received on each occasion that a food containing DHC is consumed, intakes for adults are very similar to those estimated using the concentration method (Table 11). The small difference observed is due to variability in the frequency of consumption and quantities consumer per eating occasion between individuals. When the same method is used to estimate potential intakes by children, higher values are seen because the smaller portions consumed by children are no longer taken into account.

Table 11 Potential Intakes* based on 3 mg per Eating Occasion

	DHC intake, mg/day				DHC intake, mg/kg bw/day			
Age group	Mean	P90	P95	P97.5	Mean	P90	P95	P97.5
Adults	12.4	22.3	26.1	30.4	0.2	0.3	0.4	0.4
Pre-school children	14.6	23.3	26.3	29.3	1.0	1.7	1.9	2.2
School children	13.9	21.9	24.0	26.4	0.4	0.9	1.0	1.2
Elderly	12.7	24.0	28.5	32.3	0.2	0.4	0.4	0.5

* For 'consumers' only

Regardless of the method used to assess intakes, average intake of DHC is about 12 mg/day, which represents 4 portions of food containing DHC, and high level intake is about 30 mg/day, which represents 10 portions of food containing DHC. In practice, consumers are unlikely to consume so many portions of food containing DHC in any day and so this provides further evidence for the conservative nature of this assessment.

The results of detailed assessments of potential intakes by consumers in each age group (Appendix 2.2) indicate that individuals rarely consume foods in a 'one portion per day' manner and so there is considerable variability in the daily intake. In reality, consumers might be expected to show a more regular pattern of consumption of foods containing a particular identified ingredient so that the results of this intake modelling may be somewhat unrealistic. However, given the quantities of each food category consumed by higher level consumers it can be assumed that high level intake of DHC from each food category is probably a realistic scenario. The exception might be the case of 'Vegetable protein' consumed by pre-school children. In this case it has been assumed that soya milk contains DHC because the applicant wishes to include soya-based drinks among the use applications. Apparently high intakes probably reflect children who are sensitised to cow's milk and are consuming soya milk as an alternative.

Total potential intakes (Tables 10 and 11) probably significantly over-estimate true levels of intake because it is assumed that all of food that could contain DHC actually does contain it. In reality it is likely that food manufacturers will identify only a few foods that are suitable for containing DHC and of these, consumers may not consume all of them. As a consequence the majority of potential intakes presented in this report are likely to be highly conservative because it takes account of high level consumption of all of the food categories in which DHC might be used.

Intakes from the natural occurrence of DHC in capsicums are discussed in Section III.3 of this document.

IX.2.2 Intake estimates based on the EU EFSA Concise Diets Database

The European Food Safety Authority has published a set of food consumption figures for 16 European countries. The data have been collated for 36 categories of food and data are provided separately for the entire population (i.e. including non-consumers) and for those only who consumed that food during the food consumption survey. In addition to average consumption, the database includes median and standard

deviation statistics as well as intakes the 5th, 10th, 95th, 97.5th and 99th percentiles. The data have been collected using a variety of different survey techniques which makes it difficult to make direct comparisons between countries. In particular, the duration of the survey, which could be from as little as one day to as much as a year can affect food consumption figures and the proportion of individuals reporting consumption. Nevertheless they can be used to provide a broad comparison of food consumption across these 16 European countries.

The majority of data on the consumption of processed foods to which DHC might be added as a novel food ingredient are too aggregated in the Concise Diet database to provide detailed assessments of intake. As a consequence the data have been assessed by determining the level of DHC that would be required in the broad food categories to provide 3 mg from the average amount consumed daily. This amount has been calculated for each country separately, since food manufacturers will be able to formulate products to meet the requirements of particular countries.

These use levels have then been used to estimate mean intakes from each food category on a per capita basis (i.e. including non-consumers), and mean (3 mg) and percentile intakes for consumers-only of those foods.

Total potential intakes from all foods can be calculated by summing average *per capita* intakes but cannot be calculated in the same way for percentiles because the percentile values for each food category represent different groups of consumers. High level total potential intakes can be estimated by adding the 95th percentile for each food category to the *per capita* average intake from the remainder of the diet.

The results of the intake assessment are summarised in Table 12. Average *per capita* intakes ranged from 8.3 mg/day (0.14 mg/kg bw/day) in Bulgaria up to 24.8 mg/day (0.41 mg/kg bw/day) in Norway. High level intakes ranged from 16.7 mg/day (0.28 mg/kg bw/day) in Bulgaria up to 37.9 mg/day (0.63 mg/kg bw/day) in Slovakia. The foods contributing to the high level intake are also listed in Table 12. Caution is required in interpreting high level intakes because they probably reflect foods with a high ratio between high upper percentile consumption and average consumer consumption. If it had been possible to base use levels of typical portion or serving sizes then different results would have been obtained. All of the results are conservative because they assume that consumers are consuming foods containing DHC all of the time. In reality they will probably choose only certain foods containing DHC.

Overall, the results indicate that under the most conservative set of assumptions, intakes of DHC are unlikely to exceed 40 mg/day (0.7 mg/kg bw/day for a 60 kg body-weight consumer) (Table12). They also indicate that UK consumers can be regarded as representative of typical higher level consumers across the EU.

IX.3 Food Product Labelling Information

An appropriate and clear designation (name) representing dihydrocapsiate (DHC) or formulated products containing DHC shall be displayed on the labelling of the product as such, or in the list of ingredients of foodstuffs containing it.

Approval is sought for the novel food ingredient to be available in foodstuffs throughout Europe. It is not considered that it will replace other foods.

IX.4 Conclusion on Dihydrocapsiate Intakes

Intakes of DHC from its use as a food ingredient, with use levels intended to correspond to 3 mg per adult portion are difficult to predict because individuals are likely to change their consumption patterns if they wish to consume a particular ingredient. Although it is anticipated that foods containing the novel ingredient will normally be consumed by adults, intake estimates have been developed for all potential consumers.

On the basis of current UK and European food consumption patterns, average adult intakes are unlikely to exceed 25 mg/day (or 0.4 mg/kg bw/day) and high level intakes are unlikely to exceed 40 mg/day (or 0.7 mg/kg bw/day. 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 could approach 30 mg/day (2 mg/kg bw/day). Natural levels are unlikely to contribute significantly to European intakes and so have been excluded from intake calculations.

Table 12 Potential Intakes of DHC from its use as a Food Ingredient based on EFSA Concise Diet Data from European countries

	Potential intake of DHC					
	Per capita average		High level		Food contributing highest intake	
Country	mg/day	mg/kg bw/day	mg/day	mg/kg bw/day	**Food category**	**Subcategory**
Belgium	15.97	0.27	27.25	0.45	Miscellaneous / Food for special dietary uses	Food for special dietary uses
Bulgaria	8.34	0.14	16.74	0.28	Milk and dairy based products	Dairy based products
Czech Republic	17.37	0.29	26.17	0.44	Fruit and vegetable juices, soft drinks and bottle	Soft drinks with percentage of fruits lower than n
Denmark	11.90	0.20	21.08	0.35	Fruit and vegetable juices, soft drinks and bottle	Bottled water
Finland	16.86	0.28	24.14	0.40	Miscellaneous / Food for special dietary uses	
France	18.95	0.32	29.34	0.49	Miscellaneous / Food for special dietary uses	Food for special dietary uses
Germany	23.52	0.39	35.94	0.60	Miscellaneous / Food for special dietary uses	Food for special dietary uses
Hungary	13.43	0.22	20.22	0.34	Fruit and vegetable juices, soft drinks and bottle	Soft drinks with percentage of fruits lower than n
Iceland	14.44	0.24	32.79	0.55	Miscellaneous / Food for special dietary uses	Food for special dietary uses
Ireland	17.77	0.30	25.70	0.43	Miscellaneous / Food for special dietary uses	Miscellaneous
Italy	16.64	0.28	23.65	0.39	Cereals & cereal products	Cereal-based mixed dishes
Netherlands	15.46	0.26	28.93	0.48	Vegetables, nuts, pulses including carrots, tomato	Vegetable soups
Norway	24.76	0.41	35.11	0.59	Cereals & cereal products	Cereals & cereal products excl. Cereal-based mixed
Slovakia	10.71	0.18	37.88	0.63	Miscellaneous / Food for special dietary uses	Food for special dietary uses
Sweden	13.79	0.23	23.19	0.39	Fruit and vegetable juices, soft drinks and bottle	Bottled water
UK	22.64	0.38	32.16	0.54	Fruit and vegetable juices, soft drinks and bottle	Bottled water

XI Nutritional Information on the Novel Food Ingredient

Based on the Commission Recommendation No 97/618 guidelines, the following questions must be answered in the affirmative to ensure sufficient nutritional information pertaining to the nutritional information of the novel food:

- *"Is there information to show that the novel food is nutritionally equivalent to existing foods that it might replace in the diet?"*

This question has been addressed in Section XI.1.

XI.1 Nutritional Equivalence to Existing Foods

DHC manufactured by Ajinomoto is identical to the dihydrocapsiate found as a component of the capsinoids contained in Capsicums, (Section III). It can therefore be considered to be nutritionally equivalent to the natural product and has negligible nutritional value.

XII. Microbiological Information on the Novel Food Ingredient

Based on the Commission Recommendation No 97/618 guidelines, the following question must be addressed to ensure sufficient microbiological information on the novel food:

- *"Is the presence of any microorganisms or their metabolites due to the novelty of the product/process?"*

This question is briefly addressed below and has been covered earlier in Section I.3.5 of this application.

XII.1 Microbiological Information (see Section I.3.5)

As DHC is produced by chemical synthesis under GMP with good hygienic practices and maintained under secure storage conditions using tightly sealed heavy-duty bags made of Polyethylene Terephthalate/Aluminum foil/Oriented nylon/Liner Low Density polyethylene (PET/AL/ONY/LLDPE) at -20 ºC, the possibility of any significant microbiological contamination either during production or storage is considered remote. Data on 4 Lots of Commercial Grade (Pilot Plant) DHC (Section I – Table 2) showed the absence of any pathogens, and the counts for the remaining organisms were within the range typically seen for food grade ingredients.

XIII. Toxicological Information on the Novel Food Ingredient

Based on the Commission Recommendation No 97/618 guidelines, the following questions must be addressed to ensure sufficient information pertaining to the toxicology of the novel food ingredient:

- *"Is there a traditional counterpart to the novel food that can be used as a baseline to facilitate the toxicological assessment?"*
- *"is there information from a range of toxicological studies appropriate to the novel food to show that the novel food is safe under anticipated conditions of preparation and use?"*
- *"is there information which suggests that the novel food might pose an allergenic risk to humans?"*

These questions have been addressed in Sections XIII.1 to XIII.3, respectively.

XIII.1 Is there a traditional counterpart?

There is no free, stand-alone traditional counterpart to dihydrocapsiate (DHC). Small amounts of DHC have been consumed naturally over many centuries from Capsicums due to their natural content of capsinoids which are made up of DHC, together with capsiate and nordihydrocapsiate. Singh et al (2009) reported that of 49 samples of capsicum analysed for capsiate and representing 4 cultivated species (C. annuum, var. annuum, C. annuum, var. glabriusculum, C. baccatum, C. Chinense and C. frutescens), 9 varieties contained detectable levels of DHC (18-86 µg/g tissue wet weight).

XIII.2 Toxicological and Human Health Assessment of DHC

DHC manufactured by Ajinomoto (Section II) has been extensively evaluated in a range of acute, subacute and subchronic oral toxicology studies of up to 26 weeks duration, together with genotoxicity, embryo-fetal developmental, and toxicokinetic studies. Much of the work has been published in the open literature, (Int J Toxicol, 27, Supplement 3, 2008 and Int J Toxicol, 29, Supplement 1, 2010, see Appendix 6).

Supplementary toxicological data are also discussed below that indirectly relate to DHC. These data were developed for CH-19 Sweet extract (which contains DHC) and is present in a commercial dietary supplement Capsiate Natura™ marketed by Ajinomoto in the United States of America and Japan. The encapsulated oily supplement is a refined and concentrated extract of CH-19 Sweet chilli that naturally contains capsinoids. The capsinoids consist of dihydrocapsiate (DHC), capsiate and nordihydrocapsiate which are present in CH-19 Sweet, a non-pungent cultivar of *Capsicum annuum L*. The DHC component amounts to approximately 20% of the total capsinoid content. With regard to the US product, a New Dietary Ingredient (NDI) Notification was submitted to FDA by the applicant in February 2007 (NDI, 2007). The notification was filed by the FDA without any objection on May 17, 2007. In Japan the product Capsiate Natura™ has been marketed since 2006.

The toxicology studies used a common vehicle of medium-chain triglyceride (MCT). MCT is chemically called Triglyceride C8, and the product name is Actor M-2. It was purchased from Riken Vitamin Co., Ltd. (Tokyo, Japan. The oral LD50 of MCT has been determined to be greater than 36 ml/kg, (Traul et al., 2000). The vehicle was first selected as capsinoids, the active component in CH-19 Sweet extract, are unstable in an aqueous solution but stable in an oil solution. Rapeseed oil has been used as a vehicle in some of the human safety and phamacokinetics assesment programme.

For clarity, the safety studies on the test articles DHC (commercial grade and laboratory scale) and CH-19 Sweet extract are discussed below in the following order (1) the animal and human metabolism /pharmacokinetic studies, (2) the *in vitro* and *in vivo* toxicology studies and (3) the human safety studies.

Many of the studies were originally written in Japanese and have been translated and signed by the

Technical Translator and the Study Director to confirm accurate translation. PDF versions of each study in English are contained in Appendices 3, 4 and 5. The references to each study are in the sequence: Name of the Study Director who authored the original Report, Year of the Report and Study or Experimental Number. Where a second Named reference is referred to, this relates to the Author of the International Journal of Toxicology publication in the open Literature. All identifiers are present in the Reference section.

All toxicity studies were conducted in Japan, and the principal toxicological studies in compliance with OECD guidelines. The human studies were all undertaken following internal ethical review by the Institutional Review Board (IRB) of the performing facility and in compliance with the Declaration of Helsinki (1964) and as subsequently revised.

XIII.2.1 Characterisation of DHC and CH-19 Sweet extract used for testing

The toxicological assessment of DHC was initiated by Ajinomoto using Laboratory Scale material Lot No. WKU05137ZBa. Subsequently, in 2006, due to progression from "laboratory scale" production to "pilot plant scale" production, two changes were made in the production process of the DHC precursors (i.e. MNA and V-OH) and these are described below. The core toxicology programme was thus conducted on commercial grade material produced at Pilot Plant scale following the process modifications.

1. MNA Production Change: Copper Chloride to Copper Bromide
The manufacture of MNA for the current "pilot plant scale" production process is shown in Section II.2.1. It employs copper bromide in the 'coupling reaction' whereas the initial "laboratory scale" production process employed copper chloride at this step.

2. V-OH Production Change: Methanol (as a solvent) to Tetrahydrofuran (THF)
For the current "pilot plant scale" production process of V-OH, as shown in Section II.2.1 on page tetrahydrofuran (THF) is employed as the reaction solvent. In the initial "laboratory scale" production process, methanol was employed as the reaction solvent.

The resulting Pilot Plant process was then run in order to produce 2 Lots (060807 and 070813) of Commercial Grade DHC for toxicological assessment. This material was subjected to acute, subacute, subchronic, rat and rabbit teratology/developmental studies and genotoxicity testing.

Each Lot used for toxicity testing was chemically characterized and fell within the specification shown in Table 1, Section 1. The analytical findings are shown in Table 13.

Table 13: DHC Samples used for Toxicity Testing compared with Specification

Test item	Specification (see Table 1)	Lot No.		
		WKU05137ZBa Laboratory Scale	060807 Commercial Grade	070813 Commercial Grade
Description	Viscous, colourless to yellow liquid	Viscous, pale yellow liquid	Viscous, colourless liquid	Viscous, colourless liquid
Identification (IR)	See Table 1 criteria	Conforms	Conforms	Conforms
Specific gravity	1.02-1.03	Nd	1.026	1.026
Starting Materials	V-OH Not more than 1% MNA 2-7%	0.05 2.7	<0.025 4.3	0.11 3.9
Related Substances (%)	Not more than 2%	2.02	1.39	0.55
Assay (DHC %)	≥94	95.8	94.0	95.4
Solvent (*n*-hexane) (mg/kg)	Not more than 5 mg/kg	-	<5	<5
Magnesium (mg/kg)	Not more than 1mg/kg	Nd	<0.2	<0.2
Copper (mg/kg)	Not more than 1mg/kg	Nd	<0.2	<0.2
Arsenic (mg/kg)	Not more than 1mg/kg	Nd	<1	<1
Lead (mg/kg)	Not more than 1mg/kg	-	<0.2	<0.2

Nd: Not determined

As CH-19 Sweet extract contains approximately 1.5 %w/w DHC and was subjected to detailled toxicological assessment by Ajinomoto, the product and specification are described below.

CH-19 Sweet extract is the extracted oil from CH-19 Sweet, a non-genetically modified sweet chilli pepper cultivar derived from self-progeny of CH-19 (parent cultivar), commonly eaten in Thailand. The refined and concentrated oil is a mixture of the primary capsinoids in which DHC, capsiate and nordihydrocapsiate are present in the proportions of approximately 7 :2 :1. The concentration of capsinoids in CH-19 Sweet extract is adjusted to approximately 7.5% (7.0% - 8.0%). A New Dietary Ingredient Notification of CH-19 Sweet extract was submitted to FDA by Ajinomoto Co., Inc, and the FDA provided no objection for notification of the material as a New Dietary Ingredient on May 17, 2007.

See http://www.capsiatenatura.com/fda_letter.aspx

The specification for CH-19 Sweet extract is shown in Table 14 below.

Table 14: Specification for CH-19 Sweet extract

Characteristics	Specifications	Methods
Colour	Clear yellow (at 25^{o}C)	Ajinomoto Internal Method
Odour	Peppery	smell
Appearence	Liquid	visual
Capsinoids	7.0 to 8.0%	HPLC
Water content	≤ 0.3%	Karl Fischer Titrimetric method （FCC method)
Peroxide value	≤ 10 meq/kg	Standard Methods for the Analysis of Fats, Oils and Related Materials (FCC method)
Lead	≤ 5 mg/kg	Flame atomic absorption spectrophotometric method (FCC method)
Foreign matter	Not detected	Ajinomoto Internal Method
n-Hexane	Not detected (≤ 5 ppm)	GC

FCC = Food Chemicals Codex; GC = Gas chromatography; HPLC = High Performance Liquid Chromatography; ppm = parts *per* million; **Food Chemicals Codex (2003)* 5th Edition, National Academy Press, Washington, DC

The following Lots of CH-19 Sweet extract were used in the Toxicology on the extract 041029, 050331, M050419, 050527, 050331, 050330, 050404 and 050318. All Lots conformed to specification and the individual Certificates of Analysis are attached in Appendix 1.

As additional supporting information bearing on DHC, summaries are provided in the following Sections for studies conducted employing the naturally occurring CH-19 Sweet extract. The level of DHC, the subject of this application, is identified for each study.

XIII.2.2 Metabolic fate of DHC and CH-19 Sweet extract - Animals and Man

The studies are described in priority order of relevance to assessing the toxicokinetics of the commercial grade DHC, thus the toxicology is discussed in the following order.

1. ^{14}C -DHC
2. CH-19 Sweet extract (which contains DHC at low levels)

1. ^{14}C -DHC (Appendix 3)

1.1 Pharmacokinetic studies in rats with DHC (Nemoto, H., 2005) Study No. AE-4518-G (Bernard et al. 2010).

The pharmacokinetics of ^{14}C-dihydrocapsiate were investigated by the ADME/TOX Research Institute of Daiichii Pure Chemicals Co.Ltd following a single oral gavage dose of 10 mg/11.6 MBq/kg body weight to fasting male Sprague-Dawley rats.

The plasma from 3 rats (single sample/time point) was examined at 5, 15, 30 minutes, 1, 2, 4, 6 and 8 hr after dosing. The maximal plasma concentration of the radioactivity was 1870 ng eq/ml after 0.67 hr and then declined with an apparent half-life of 2.4 hr. The AUC was 7581 ng eq·hr/ml. Most of the radioactivity was rapidly eliminated within 48 hours, with 98.1% of the radioactivity excreted after 72 hr, 78.2% in the urine, 19.4% in the feces and 0.5% in expired air, respectively. The carcass residue was 4.0% of the dose at this time.

After single oral administration to fasting male rats the bile, urine, faeces, gastrointestinal contents and carcass (single sample/time interval or time point) were collected and examined (in 3 rats) at the following intervals or time points: bile (0 - 2, 2 - 4, 4 - 8, 8- 24 and 24- 48 hr after dosing), urine and feces (0 - 24 and 24- 48 hr after dosing), GI contents (48 hr after dosing), carcass

(excluding GI contents, 48 hr after dosing). The excretion of radioactivity in the bile, urine and feces were 3.4%, 68.2% and 6.0% of the dose up to 48 hr after dosing, respectively. The residual radioactivity in the GI contents and carcass were 5.6% and 14.3%, respectively. This indicates that the urine is the major route of excretion with limited excretion via the bile.

Tissue distribution data was obtained from three fasted male rats (25 tissues examined/rat) at each of 5 time points (15 and 30 minutes, 2, 6 and 24 hr) after single oral administration. The radioactivity concentrations in the tissues reached maxima at 30 minutes in the fat and small intestines, at 6 hr in the stomach and large intestine and at 2 hr in the other tissues. The radioactivity concentrations which were higher than that in the plasma were observed in the kidney, liver, stomach, small intestine and large intestine. At 6 hr, plasma levels had decreased to 15% of the maximum value at 2 hr, and all tissue levels (except digestive tract) had decreased. The concentration of radioactivity in the kidney (the main organ of excretion) was the highest, being 6.49 times that in the plasma. At 24 hr, the radioactivity had decreased further with the plasma reaching 2% of its maximum.

Analysis for plasma DHC concentrations showed that 15 minutes after dosing, DHC was below the limit of detection (LOD), while main metabolites RP2, RP3 and RP4 accounted for 23.8%, 46.4% and 2.8% of the radioactivity in the plasma, respectively. In the plasma at 30 minutes and 2 hr after dosing, DHC was again not detected while RP2 and RP3 accounted for 15.5% and 49.4% of the radioactivity in the plasma, respectively. In the plasma at 6 hr after dosing, DHC was not detected, while RP2 and RP3 accounted for 21.4% and 20.8% of the radioactivity, respectively. On the HPLC radiochromatograms of the 30 minutes and 2hr plasma after treatment with β-glucuronidase/arylsulfatase, RP2, RP3 and RP4 disappeared, whereas vanillyl alcohol and vanillic acid were detected. Hydrolysis of only RP2 was inhibited by addition of β-glucuronidase inhibitor, suggesting that RP2 is a glucuronide of vanillyl alcohol, whereas RP3 is a sulfate of vanillyl alcohol, and RP4 is a sulphate of vanillic acid. (Bernard et al., 2010)

Radioactivity in the plasma was associated with several DHC metabolites and their conjugates while DHC was below the limits of detection. The metabolites were found to be vanillyl alcohol, vanillic acid, vanillyl alcohol glucuronide, vanillyl alcohol sulphate, and vanillic acid sulphate.

Conclusion on Toxicokinetics of DHC

In conclusion this study showed that the highest tissue concentrations after oral dosage are found in the major organs/systems of absorption, metabolism and excretion, namely the, GI tract, liver and kidney. DHC is metabolized by hydrolysis in the gut, the metabolites are rapidly absorbed and conjugated in the liver and predominantly eliminated by the kidneys into the urine. The rapid absorption, short half-life and high level of excretion shows that accumulation of DHC or its metabolites in the tissues is unlikely to occur.

2. CH-19 Sweet extract (Appendix 5)

2.1 Metabolism and pharmacokinetic studies in rats with CH-19 Sweet extract (Capsinoids). (Shirai Y., 2005a) Study No. XX05E-1103 and (Shirai Y., 2005b) Study No. XX05E-1002, (Shirai Y et al., 2008).

Ajinomoto Co., Inc., Drug Metabolism and Pharmacokinetics Department, Japan investigated the metabolism and pharmacokinetics of CH-19 Sweet extract containing approximately 7.5% of capsinoids (DHC, capsiate and nordihydrocapsiate) administered by a single oral gavage dose to male Sprague-Dawley rats. Two doses (10 and 100 mg capsinoids/kg) approximately equivalent to 2 and 20 mg DHC /kg were studied. In the first study (XX05E-1103) conjugation was studied in plasma and a tendency was observed for higher concentrations of the vanillyl alcohol sulphate than the glucuronide at each time. In the second study (XX05E-1002) levels of capsinoids and vanillyl alcohol (a major metabolite) were measured in portal vein and abdominal aorta blood in order to determine the concentration-time profile in rats after single oral gavage administration. Capsinoids (DHC, capsiate and nordihydrocapsiate) were not detected at any time points examined in both 10 and 100 mg/kg dose groups indicating rapid metabolism to the vanillyl alcohol, a common metabolite for each of the 3 capsinoids. Vanillyl alcohol in the portal vein plasma attained maximum plasma concentrations (C_{max}) of 0.163 µg/ml and l.48 µg/ml 30 minutes after administration to the 10 and 100 mg/kg dose groups, respectively. Areas under the portal vein plasma-concentration time curves of vanillyl alcohol (AUC 0-4hr) were respectively

0.321 µg·hr/ml and 3.85 µg·hr/ml after doses of 10 and 100 mg/kg respectively. Vanillyl alcohol was not detected in the systemic plasma at any time points following dosing with 10 mg/kg whereas low concentrations (0.0246 µg/ml were detected 5 minutes after dosing with 100 mg/kg but at no time points thereafter.)

2.2 Studies to determine any effect of capsinoids on liver cytochrome P450 (Takahashi, 2005) Study No. XX05E-104 (Takanohashi et al. (2009).

The potential for inhibitory activity on the mixed function oxidase CYP3A4 of the capsinoids (DHC, capsiate and nordihydrocapsiate) and capsaicin was examined in the Drug metabolism & Phamacokinetics Department of Ajinomoto Co., Inc., Japan. DHC, capsiate, and nordihydrocapsiate were dosed at a concentration of 100µmol/L and showed no inhibitory activity whereas capsaicin demonstrated a significant inhibitory effect on CYP3A4, having an IC50 value of 21.5 µmol/L.

2.3 Single dose pharmacokinetic studies in human male volunteers with CH-19 Sweet extract (capsinoids) (Nakamura, T., 2006) Study No. PBC043-011 2006, and (Hamada, M., 2006) Study No. FO603 & (Bernard et al., 2008e)

The pharmacokinetics of CH-19 Sweet extract were investigated by Shin Nippon Biomedical Laboratories Ltd, Japan, using blood samples taken from 16 healthy male volunteers on clinical Study No. FO603. The protocol involved a single oral dose using soft gel capsules containing either capsinoids (15 or 30 mg/person) approximately equivalent to 3.96 or 7.92 mg DHC/person or placebo. Rapeseed oil was used as the diluent and also the vehicle for the gel capsules. Blood samples were collected at 15 and 30 min, 1, 2, 4, 8 and 24 hr after ingestion of the test article. Plasma concentrations of capsinoids (i.e., DHC, capsiate and nordihydrocapsiate) and vanillyl alcohol were analysed but were found to be below the limit of quantification (10 ng/ml) for DHC, capsiate, nordihydrocapsiate and below the LOD (50 ng/ml)l for vanillyl alcohol) at all time points examined. The results are derived from a sub-population of the volunteers on a combined safety and pharmacokinetic study (Study No. F0603) conducted at Kurume Clinical Pharmacology Clinic.

Conclusion on Toxicokinetics of CH-19 Sweet extract

In conclusion, the results from the studies conducted indicate that capsinoids contained in the orally administered CH-19 Sweet extract are metabolized in the gastrointestinal tract or gut mucosa (or both) before absorption. The absorbed vanillyl alcohol found in the portal vein is subjected to metabolic conversion by sulphation and glucuronidation during its passage through the liver before entering into the post-hepatic systemic blood. Absorption and metabolism are thus seen to be rapid and extensive following oral administration to rats and man. There was no evidence for inhibitory effects on the mixed function oxidase CYP3A4.

XIII.2.3 Toxicological Evaluation of DHC (Animal Studies)

The studies are described in priority order of most relevance to assessing the toxicology of the commercial grade DHC, thus the toxicology is discussed in the following order.

1 Commercial Grade DHC
2 Laboratory Scale DHC
3 CH-19 Sweet extract (which contains DHC)

Many of the original reports summarised below refer to the testing of “ASDCT, dihydrocapsiate”. At the time of the studies the Company notation for the test article was for “Ajinomoto Substance DihydroCapsiaTe” ie ASDCT. To prevent the risk of confusion the technical terms dihydrocapsiate or DHC are used exclusively for dihydrocapsiate (DHC) throughout this application.

1. Toxicology on Commercial Grade DHC (Appendix 3)

An extensive programme of testing has been conducted on the commercial grade DHC material, see Table 15 below

Table 15: Summary of Toxicity Studies Conducted on Commercial Grade DHC

ID	Study Type	Lot No.	Author (SD) Study/Exp't No.	Result
1.1	Oral acute limit toxicity test in mice	060807	Kodama T. 2007 07A003	LD_{50} >5000 mg/kg
1.2	13-week oral gavage toxicity study in rats	060807	Ohishi T. 2007 C-B303	NOAEL >1000 mg/kg
1.3	26-week oral gavage toxicity study in rats	070813	Ohishi T. 2007/9 C-B373	NOAEL >1000 mg/kg
1.4	Oral gavage teratology and developmental toxicity study in rats	060807	Ikeya M. 2007 CR060	Maternal and Fœtal NOAEL >1000 mg/kg
1.5	Oral gavage teratology and developmental toxicity study in rabbits	060807	Matsuoka T. 2007 C-R061	Maternal and Fœtal NOAEL >1000 mg/kg
1.6	Gene mutation in transgenic rats	060807	Nakajima M. 2007 9994 (258-062)	Not mutagenic
1.7	Mouse micronucleus test	060807	Nakajima M. 2007 A673 (258-066	Not clastogenic

ID – Sequence Identification in Section
SD- Study Director/Author of Original Study Report

1.1 Oral acute limit toxicity test in mice (Kodama T. 2007) Study No. 07A003 (Watanabe et al., 2008b)

An acute oral gavage toxicity study was performed in 2007, in the Ajinomoto Co., Inc., Research Laboratories, according to the US-Redbook 2000 Guidelines. The study was performed to Ordinance No. 21 (March 26, 1997) on the standard for the conduct of non-clinical studies on safety of drugs, the Ministry of Health and Welfare, Japan.

The study was carried out on male and female ICR mice (Crj/CD-1(ICR). After a 14 days acclimation period, 5 mice of each sex (6 weeks old, mean body weight of 29.7 g and 21.4 g for males and females respectively) were randomly allocated to each treatment group. The test substance (DHC, lot 060807, 94.0% pure) was diluted in a vehicle (medium chain triglyceride: MCT) and administered at a dose of 5000 mg/kg. A control group received the vehicle alone.

During the 2 hour post-dose observation period, staggering gait, decreased spontaneous movement, prone position, tremor, gasping or red-brownish urine were observed in males and females in the treated group. These findings were transient and had resolved by 6 hours post-dosing. No deaths, body weight changes or abnormal gross pathological findings were observed at the end of the study.

Because all the animals survived, the oral LD_{50} in mice was concluded to be higher than 5000 mg/kg.

1.2 13-week oral gavage toxicity study in rats (Ohishi T. 2007) Study No. C-B303 (Watanabe E., 2008d)

A 13-week subchronic oral gavage toxicity study of DHC was performed in 2007, by the Bozo Research Center Inc. The study 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).

The study was carried out in male and female Sprague Dawley (Crl:CD(SD) rats. Forty rats of each sex were selected (6 weeks old, mean body weight of 217 g and 155 g for males and females respectively) and 10 of each were randomly allocated to each treatment group.

The test substance (DHC, lot 060807, 94.0% pure) was diluted in a vehicle (medium chain triglyceride: MCT) and administered daily by gavage at 100, 300 and 1000 mg/kg. The controls received vehicle alone.

No death or abnormal clinical signs occurred during this study and there were no statistically significant differences in bodyweight. Slight increases in food consumption and sporadic increases in water intake occurred in the male high-dose group during the second half of the study. No changes in the ophthalmology or haematology were observed which could be related to administration of the test article.

Blood chemical analyses revealed a marginal increase in ALT activity in the high-dose group mean in both sexes, but this was within the historical range for the laboratory and quantitatively marginal 8 and 5 IU/L (greater than the corresponding control), in males and females respectively. Total protein was also increased male high-dose animals. Males in this group showed a significant decrease in creatine which was determined not to be of toxicological significance. Males in the high-dose group demonstrated an increase in urine volume and excretion of sodium, potassium and chloride. As these parameters were not significantly altered in the blood; the observations in the urine were considered unlikely to be of toxicological significance.

High-dose males demonstrated a statistically significant but slight increase in absolute and relative liver and kidney weight. High-dose females demonstrated a similar effect in relative liver and kidney weight. However, there were no corresponding histopathological findings with the exception hepatocyte hypertrophy (graded minimal in one rat and mild in the other) in 2 of the high-dose males. Other histopathological changes were determined to be incidental or spontaneous being frequently observed in untreated animals of this strain of rats.

Based on the lack of evidence for any toxicity, the no-observed-adverse-effect level (NOAEL) is considered to be 1000 mg/kg/day for males and females. Nevertheless it should be noted that the original report from Bozo regarded the NOAEL in males to be 300 mg/kg/day based on the minimal hepatic response. While it can be argued whether this represented a frank adverse effect or not, a subsequent 26 week oral gavage study (described below – Study No. C-B373 considered the NOAEL for both sexes to be 1000 mg/kg/day). Thus doubling the duration of the study does not appear to result in more than an adaptive, non adverse, hepatic response to the high dosage level administered. This is reassuring that 1000 mg/kg/day may be interpreted as the NOAEL for both sexes in this 13 week oral gavage study.

1.3 26-week oral gavage toxicity study in rats (Ohishi T. 2007 & 2009) Study No. C-B373 and C-B373 (Amendment-1) (Kodama T, et al 2010)

A 26-week oral gavage toxicity study of DHC followed by a 4-week recovery period was performed in 2007 by the Bozo Research Center Inc, Japan.

The study was conducted 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).

The study was carried out in male and female Sprague Dawley (Crl:CD(SD) rats. Eighty rats of each sex were selected (6 weeks old, mean body weight of 229 g and 170 g for males and females respectively) and 20 of each sex were randomly allocated to each treatment group. In addition, satellite groups of 10 animals of each sex received either 0 or 1000 mg/kg DHC and were designated for a 4 week recovery period following the cessation of dosing after 6 months.

The test substance (DHC lot 070813, 95.4% pure) was diluted in a vehicle (medium chain triglyceride: MCT) and administered daily by gavage at 100, 300 and 1000 mg/kg. The control group received the vehicle alone.

No rats died during the course of the study but one high dose male was found moribund on day 127. Necropsy showed a cerebellar nodule which, when followed by histopathology was found to be an oligodendroglioma in the cerebrum. This was judged to be a spontaneous finding unrelated to treatment. Two high females presented with subcutaneous masses in the neck or axilliary region and the animals survived to term. The other animals appeared normal throughout the study.

Mean body weight and weight gain were unaffected by treatment. Food consumption was higher in high-dose females compared to the control group from day 77 of treatment but other groups were unaffected. Water consumption was higher in the high-dose groups and remained higher in the high-dose females during the recovery period. Some statistical variations, which were

quantitatively minimal, were seen in the haematology. As these remained within the normal historical control ranges for the performing laboratory they were not considered to be adverse. Small, within normal limits increases in ALT were seen in mid and high dose animals of either sex, together with increased total cholesterol in high dose males. There were no changes in the other liver enzymes (AST and LDH and ALP) and histopathological examination of the liver revealed no evidence of toxicity. An increase of the liver weight and the presence of periportal hepatocyte hypertrophy in 2 high dose males and 3 high-dose females in the absence of necrosis was not considered to be of toxicological significance.

Statistically significant higher values in the absolute and relative weight in the pituitary were observed in females in the high dose group at the end of the recovery period together with higher values in females for salivary gland weight (absolute), adrenal weight (absolute) and liver weight (relative). Statistically significant increases in both the incidence of kidney tubular regeneration and kidney urinary cyst hyaline were observed in high dose females at the termination of dosing after 26 weeks. At the end of the recovery period, two adenocarcinomas and increased incidence of mammary gland focal or lobular hyperplasia were observed in high dose females and also in controls. These findings were judged to be incidental or spontaneous as Sprague-Dawley rats commonly develop age related, pituitary hormone dependent, proliferative changes in the mammary gland. The histopathology was consistent with this. Of the 12 rats observed to have proliferative changes in the mammary gland (3 males and 9 females), 4 were seen in the control group and 8 in the high dose group. Ten of the twelve animals had weights that exceeded their respective group means. There were no treated related changes in the histopathological findings of the pituitary.

Based on the results of this study, the 26-week gavage NOAEL for DHC is 1000 mg/kg for both male and female rates.

1.4 Oral gavage teratology and developmental toxicity study in rats (Ikeya M., 2007) Study No. C-R060 (Bernard BK, 2008b)

An oral gavage teratology study in rats was performed on DHC in 2007 by the Bozo Research Center Inc, Japan.

The study 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).

The study was carried out in female Sprague Dawley (Crl:CD(SD) rats. Eighteen to 20 female rats (11 weeks old, mean body weight of 223 g) were randomly allocated to each treatment group.

The test substance (DHC, lot 060807, 94.0% pure) was administered by gavage using (medium chain triglyceride: MCT) vehicle. Rats were dosed for 11 consecutive days from day 7, (ie between days 7-17 of gestation inclusive) at 100, 300 and 1000 mg/kg. Controls received the vehicle alone.

There were no deaths or dose-related changes in the dams, including clinical signs, body weight gain, food consumption or gross pathology. Moreover there were no abortions or premature births. Macroscopic examination revealed no abnormalities in any major organs/tissues in the abdominal or thoracic cavity in any group.

At the end of the gestation period caesarean section showed that the number of corpora lutea, number of implantations, and implantation index were within normal limits as were indices for embryo-foetal death, number of live foetuses, sex ratio and body weight. The placentas appeared normal in the animals from each group.

No trends or dose-related changes were observed during detailed external examination and sexing of the live foetuses. Foetuses with external abnormalities were weighed, fixed, and preserved in phosphate-buffered 10% formalin. For each litter approximately half the live foetuses, except those with abnormalities, were fixed in Bouin’s solution. Internal organs were examined for visceral abnormalities/variations using Wilson’s technique (Wilson, 1965) for the cephalic cavity and a microdissection method for the thoracic and abdominal cavities (Nishimura, 1974). The remaining foetuses were prepared for skeletal examination using a modified

Dawson's method, (Dawson, 1926). The incidence of almost all the external, skeletal and visceral examinations were within the background data of the test facility and there were no increases that appeared to be related to the administration of DHC. There were no test substance-related effects on the development or growth of foetuses or changes in any index for the progress of ossification: the numbers of ossified metacarpi, ossified metatarsi, and ossified sacral and caudal vertebrae, and the index of ossified sternebrae.

Based on these results, it was concluded that the test substance has neither teratogenic, developmental nor growth effects on the foetuses.

The no-observed-adverse-effect level (NOAEL) in rats was considered to be 1000 mg/kg/day for the dams, for pregnancy and for embryo-foetal development. There was no evidence of teratogenic potential.

1.5 Oral gavage teratology and developmental toxicity study in rabbits (Matsuoka T. 2007) Study number No. C-R061 (Bernard BK, 2008b)

A rabbit teratology study dosed orally by gavage was carried out in 2007 by the Bozo Research Center Inc.

The study 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).

The study was carried out in female New Zealand White SPF rabbits (Kbl:NZW Kitayama Labs Co.Ltd, Nagano, Japan). Animals (16-17 weeks old) were mated and randomised into 4 groups with twenty two rabbits per group. The test substance (DHC, lot 060807, 94.0% pure) was administered in a vehicle (medium chain triglyceride: MCT) and administered daily by gavage for 13 days from day 6 of gestation at 100, 300 and 1000 mg/kg. Controls received the vehicle alone.

There were no deaths, premature births or abortions in the dams. The number of non-pregnant rabbits was acceptable being 1,1,3,1 in the control, low, mid and high dosage groups. Between days 24-28 of gestation, 1 to 3 animals/group, including controls, showed reduced faeces. This finding was judged to be incidental as none of the animals were being dosed at that time and there was no dose relationship. There were no other clinical signs and body weight gains, food consumptions and necropsy findings were all within normal limits for this strain of rabbit.

At the end of the gestation period, following caesarean section on day 28, there were no significant differences in the number of corpora lutea, number of implantations, or implantation index. With regard to embryo-foetal development, there were no significant differences that were considered to be treatment related between test and control animals with regard to the number or indices of embryo-foetal death, the number or sex ratio of live foetuses, their body weight or in the gross pathology of the placentas.

No trends or dose-related changes were observed during detailed external examination and sexing of the live foetuses. Three foetuses were found to have minor external abnormalities. For all foetuses that appeared normal at caesarian section, internal organs in the thoracic cavity (except internal observation of the heart) and those in the abdominal cavity were observed macroscopically for abnormalities and the sex of the rabbit confirmed. The brain and heart were removed, fixed in phosphate-buffered 10% formalin, and examined for dilatation of the brain using Wilson's method and for visceral abnormalities using Nishimura's microdissection method. After observation, visceral organs/tissues were preserved in phosphate-buffered 10% formalin. The remaining foetuses, with the exception of the 3 mentioned above with external abnormalities, were prepared for skeletal examination by removing the skin and fatty tissue from fresh specimens, fixing the foetuses in 95% alcohol and staining them using Dawson's method, (Dawson, 1926). After the end of observation, the specimens were preserved in 50% glycerine solution (containing 0.5 mg/ml thymol).

No dose-related changes were noticeable following external, skeletal and visceral examinations of foetuses including the progress of ossification. Of the 3 foetuses observed with external abnormalities, the first was a control with hypoplasia of the body and face, thoracogastroschisis, meningoencephalocoele, spina bifida and ectrodactyly of the first digit of the right fore limb. The second abnormality was observed in one foetus from each of the mid and high dose groups and

involved bilateral paw hyperflexion in the fore limb or hind limb respectively. These findings were not dose related and were judged to be incidental in nature.

Based on these results, it was concluded that the test substance has neither teratogenic, nor developmental (growth/maturation) effects on the foetuses.

The no-observed-adverse-effect level (NOAEL) was judged to be 1000 mg/kg/day in both dams and foetuses.

1.6 Gene mutation in transgenic rats (Nakajima M., 2007) Experiment No. 9994 (258-062) (Bernard BK., 2008a)

A gene mutation assay of DHC in transgenic Big Blue^{TM} rats was carried out in 2006 by the Biosafety Research Center, Shizuoka, Japan, according to the recommendation of the WHO-IPCS (2006).

The assay was performed on male Big Blue^{TM} rats (Fisher 344 [SPF]) aged 8 weeks. Five animals per group were dosed orally by gavage at 500 and 1000 mg/kg for 28 consecutive days. The test substance (DHC, lot 060807, 94.0% pure) was diluted in medium chain triglyceride (MCT) that was also used as negative control. The positive control used was: 7,12-dimethylbenzo- (a)anthracene (DMBA).

No deaths, clinical signs or changes in bodyweight occurred during the study. After 3 days following the final treatment, duodenum, liver, and kidney were removed and analyzed for mutant frequency. The organs (respectively of absorption, conjugation and excretion) were selected based on toxicology studies described previously.

Duodenum

The negative control demonstrated a mutant frequency of 43.5 x 10^{-6}. The mean among individuals was 44.2 x 10^{-6}. Mutant frequencies of 36.8 x 10^{-6} and 32.0 x 10^{-6} were observed in the low- and high-dose groups respectively. The means among individuals in the low and high-dose groups were 35.7 x $l0^{-6}$ and 32.3 x 10^{-6}, respectively. The findings in the treatment groups were comparable to the negative control group.

The positive control group exhibited a mutant frequency of 237.9 x $l0^{-6}$, which was statistically significant as compared to negative controls. The mean individual mutant frequency was 237.5 x $l0^{-6}$.

There were no statistically significant differences between the treatment groups as compared to the negative control group.

Liver

The negative control demonstrated a mutant frequency of 35.7 x 10^{-6} (mutant plaques out of total plaques). The mean among rats was 36.7 x 10^{-6}. Mutant frequencies of 24.5 x 10^{-6} and 28.1 x $l0^{-6}$ were observed in the low- and high-dose groups respectively and were comparable to the negative control group. The means in the low- and high-dose groups were 24.0 x 10^{-6} and 28.2 x $l0^{-6}$, respectively. The findings in the treatment groups were comparable to the negative control group.

The positive control group exhibited a mutant frequency of 118.3 x $l0^{-6}$, which was statistically significant as compared to negative controls. The mean for individuals was 115.2 x 10^{-6}.

Kidney

The negative control demonstrated a mutant frequency of 22.5 x 10^{-6}. The mean among individuals was 22.3 x $l0^{-6}$. Mutant frequencies of 28.7 x 10^{-6} and 30.1 x 10^{-6} were observed in the low- and high-dose groups respectively. The means among individuals in the low- and high-dose groups were 29.7 x 10^{-6} and 30.1 x $l0^{-6}$, respectively. The findings in the treatment groups were comparable to the negative control group.

The positive control group exhibited a mutant frequency of 75.8 x 10^{-6}, which was statistically significant as compared to negative controls. The mean among the individuals was 82.7 x 10^{-6}.

In conclusion, no statistically significant increase in mutant frequency was observed in the liver, kidney or duodenum of any groups. Under the conditions of this study, DHC was not considered to be mutagenic.

1.7 Mouse Micronucleus test (Nakajima M., 2007) Experiment No. A673 (258-066) (Watanabe E., 2008b)

An *in vivo* micronucleus study was carried out on DHC in 2007 by the Biosafety Research Center (Shizuoka, Japan). This assay was conducted referring to Ordinance No.21 (March 26, 1997) on standard 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 (DHC, lot 060807, 94.0% pure) was diluted in medium chain triglyceride (MCT) that was also used as negative control. Mitomycin C (MMC), 0.5 mg/kg intraperitoneally, was used as the recommended positive control and induced a marked statistically significant increase in micronucleated cells compared with control.

For the test substance, DHC was administered by oral gavage at doses of 500, 1000 and 2000 mg/kg for 2 days. No deaths or decreases of bodyweight occurred during the study and there were no changes in the ratio of bone marrow micronucleated polychromatic erythrocytes MNPCEs) compared with the negative control group.

Under the conditions of this assay, DHC was considered as not clastogenic.

Discussion and Conclusion on Toxicology of Commercial Grade DHC

From the above studies on Commercial Grade DHC, it can be seen that the substance has a low acute oral toxicity (> 5,000 mg/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 with an overall NOAEL across different studies of 1000 mg/kg bw/day. The only consistent changes seen were in the subacute and subchronic rat studies where a probable weak physiological response to dosage, at the limit dose of 1000 mg/kg, resulted in slight weight increases in the in the organs of metabolism (liver) and excretion (kidney) but in the absence of toxicity as evidenced by the histopathological examination. Small changes in ALT were also seen in individual animals but these were generally within normal limits for the age and sex of rats involved and for the Contract testing facility. Only 2 high dose-level male rats in the 13 week study (and none in the 6-month study) showed either minimal or mild grade hepatocellular hypertrophy. In consequence, the high dosage level in both the 13 and 26 week repeat dose studies was judged to be without toxic effect, and hence the NOAEL was considered to be 1000 mg/kg.

2. Toxicology on Laboratory Scale DHC (Appendix 4)

The early laboratory scale material was subjected to initial toxicological screening involving subacute, and genotoxicity studies. These are summarised in Table 16 below.

Table 16: Summary of Toxicity Studies Conducted on Laboratory Scale DHC

ID	Study Name	Lot No.	Author (SD) Study/Exp't No.	Result
2.1	13-week oral gavage toxicity study in rats	WKU05137ZBa	Mochizuki M. 2006 N-B205	NOAEL >1000 mg/kg
2.2	Bacterial reverse mutation test	WKU05137ZBa	Shimada S. 2006 9612 (258-046)	Non-mutagenic +S9 Mutagenic in TA100 only in absence of S9
2.3	*In vitro* chromosome aberration test	WKU05137ZBa	Masumori S. 2006 9613 (258-047)	Non-clastogenic +S9 Clastogenic only in absence of S9
2.4	*In vivo* mouse micronucleus test	WKU05137ZBa	Nakajima M. 2006 9623 (258-048)	Non-clastogenic
2.5	*In vivo* Comet assay in rats	WKU05137ZBa	Shimada S. 2007 9993 (258-061)	Equivocal DNA damage, within historical control values

ID – Sequence Identification in Section SD- Study Director/Author of Original Study Report

2.1 13-week oral gavage toxicity study in rats (Mochizuki M. 2006c) Study No. N-B205 (Kodama T et al., 2008a)

A 13-week subchronic oral gavage study of DHC was performed in 2006, by the Bozo Research Center Inc. The study 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).

The study was carried out in male and female Sprague Dawley Strain (Crl:CD(SD) rats. Forty rats of each sex were selected (6 weeks old, mean body weight of 222 and 165 g for males and females respectively) and 10 of each were randomly allocated to each treatment group.

The test substance (DHC, lot WKU05137ZBa, 95.8% pure) was administered in a vehicle (medium chain triglyceride, MCT) at 100, 300 and 1000 mg/kg. The control group received the vehicle alone.

There were no deaths and no clinical signs which could be related to treatment. No dose-related changes were observed for body weight, body weight gain, food consumption or water intake. None of the variations in ophthalmology, urinalysis and haematology were considered to be related to administration of the test substance as they were either within the normal range or showed no dose relationship. The blood chemistry showed some statistically significant differences at the highest dose level for both males and females in total proteins, for males only, in ALT activity and for females only, in calcium, albumin, A/G ratio, γ-globulin level. The changes, with the exception of the ALT increase in high dose males (Control 27 IU/L versus High Dose 52 IU/L), were found to be within the control range taking when checking the individual values. Looking at the increase in ALT activity, the effect in high dose male rats was soley due to a high value (196 IU/L) in a single male rat (4010M) that exceeded the range of the control values (18-63 IU/L). Organ weight analysis revealed a treatment related increase of the liver weights, both absolute and relative, in high dose animals of either sex, which in the absence of any histopathological findings were considered to be adaptive (physiological) in nature. Histopathological examination of the liver from rat 4010 with the high ALT value showed no test substance-related changes. It was thus concluded that it was unclear whether the increased ALT value in the affected rat was an incidental finding or due to a physiological (adaptive) response. However in the absence of histopathological or other enzyme changes in the liver of this animal or any similar changes in the remaining animals of this group or in the female high-dose, the finding was not considered to be of toxicological significance.

No histopathological changes were observed in any of the other organs examined.

In conclusion, based on the findings, the no-observed-adverse-effect level (NOAEL) was 1000 mg/kg/day for males and for females.

2.2 Bacterial reverse mutation test (Shimada S. 2006) Experiment No. 9612 (258-046) (Bernard BK et al., 2008a)

A reverse mutation test using *Salmonella typhimurium* and *Escherichia coli* was carried out in 2006 by the Biosafety Research Center (Shizuoka, Japan), according to according to the Japanese guidelines of the Industrial Safety and Health Law (notification based on Article 57-3-1 of the Industrial Safety and Health Law (Ordinance No. 57, 1972).

Four *Salmonella typhimurium* strains (TA98, TA100, TA1535 and TA1537) and one *Escherichia coli* strain (WP2uvrA) were assayed with and without metabolic activation (S9 mix). The S9 was prepared from the liver of Sprague-Dawley rats treated with phenobarbital (up to 60 mg/kg ip) and 5,6-benzoflavone (80 mg/kg ip).

The test substance (DHC, lot WKU05137ZBa, 95.8% pure) was diluted in dimethyl-sulfoxide (DMSO) that was also used as negative control. Recommended positive controls were assayed: Sodium azide (NaN3), 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2); 9-aminoacridine (9-AA) and 2-aminoanthracène (2-AA).

In the preliminary range-finding cytoxicity assay, up to 5000 µg/plate was tested in the presence or absence of S9 mix but this led to precipitation at the highest dose tested and growth inhibition above 300 in the plates evaluated without metabolic activation and above 800 µg/plate with 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 100µg/plate. There was no evidence for any effect in the DHC treated plates that included metabolic activation with S9 mix. The positive controls gave a pronounced increase in revertant colonies both with and without metabolic activation.

In the definitive assays, up to 1000 and 2500 µg/plate were tested without S9 and with S9 respectively. Except for TA100 without S9 mix where there was a dose dependent two fold or more increase in the number of revertant colonies compared with control, no mutagenic activity was observed with or without metabolic activation for any of the other tested strains. A doubtful 1.77 fold response was seen at 500 µg/plate with TA 98 in the –S9 assay where growth inhibition occurred; there was no effect at 1000 µg/plate, and the 2-fold threshold was not achieved. No similar effect was seen in the range-finding study.

Under the condition of this assay, DHC was found not to be mutagenic in any of the tester strains in the presence of metabolic activation, although TA 100 showed a response in the absence of S9 only.

2.3 *In vitro* chromosome aberration test (Masumori S. 2006) Experiment No. 9613 (258-047) (Bernard BK et al., 2008a)

A mammalian cell chromosomal aberration study was carried out in 2006 by the Biosafety Research Center (Shizuoka, Japan), according to the Japanese guidelines of the Industrial Safety and Health Law (notification based on Article 57-3-1 of the Industrial Safety and Health Law (Ordinance No. 57).

CHL/IU cells from Chinese hamster lung fibroblast were assayed with and without metabolic activation (S9 mix). The S9 was prepared from the liver of Sprague-Dawley rats treated with phenobarbital (up to 60 mg/kg ip) and 5,6-benzoflavone (80 mg/kg ip).

The test substance (DHC, lot WKU05137ZBa, 95.8% pure) was diluted in dimethylsulfoxide (DMSO) which was also used as negative control. Mitomycin C (MMC) and cyclophosphamide (CP) were used as positive controls.

The dose for the chromosome aberration test was determined based on the results of a preliminary cell growth inhibition test where up to 3084 µg/ml was assayed with and without S9 mix for both short term and continuous treatment. DHC showed cytotoxic action in each treatment system.

For the definitive chromosome aberration study, up to 324 µg/ml were assayed for tests without S9 mix and continuous treatment. Up to 1500 µg/ml was assayed for short term exposures with S9mix. Doses above 194 µg/ml in the –S9 short and long term studies could not be used for metaphase analysis due to evident cytotoxicity and hence the lack of metasphase cells. Microscopic examinations were performed on the mitotic cells at 3 dose levels in each treatment as follows: 70.0, 117, and 194 µg/mL for the short-term treatment -S9 assay, 540, 900, and 1500 µg/ml for the short-term treatment +S9 assay, and 70.0, 117, and 194 µg/ml for the continuous treatment 24-hour assay.

The results showed that in each dosage treated with DHC in the short-term treatment +S9 assay the incidences of cells with chromosome aberrations (structural aberrations and numerical aberrations) were less than 5%, and that 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 only observed at 194 µg/ml with figures of 9.0% and 8.0%, respectively which was considered inconclusive. Moreover, 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 continuous treatment, 24-hour, assay, an increased incidence of cells with chromosome structural aberrations only 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%, it was judged positive. Therefore, to confirm the reproducibility or the dose-dependency for the short-term treatment -S9 assay and the continuous treatment 24-hour assay, further assays were performed on the mitotic cells at the 3 dose levels of 96.0, 137, and 196 µg/ml in each treatment and the positive

response was confirmed. Both positive controls, resulted in significant increases in chromosomal aberration.

Under the condition of this assay, it was concluded that DHC is not clastogenic in the presence of metabolic activation but appears to be in the absence. These results indicate that the metabolites of DHC have no apparent mutagenic or clastogenic activity whereas the parent compound DHC may do.

2.4 *In vivo* mouse micronucleus test (Nakajima M., 2006) Experiment No.9623 (258-048) (Bernard BK et al., 2008a)

An *in vivo* micronucleus study was carried out in 2006 by the Biosafety Research Center (Shizuoka, Japan). This study was conducted referring to Ordinance No. 21 (March 26, 1997) on standard 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 (C57BL/6 x DBA/2) aged 9 weeks. Five animals per group were used. The test substance (DHC, Lot WKU05137ZBa, 95.8% pure) was diluted in medium chain triglyceride (MCT) that was also used as the negative control. The recommended positive control mitomycin C (MMC) was employed.

The test substance was administered by oral gavage (0.5ml/100g BW) and from the preliminary dose range-finding study 2000 mg/kg was selected as the high dose level. In consequence the main micronucleus study was treated by gavage at doses of 500, 1000 and 2000 mg/kg for 2 consecutive days.

No deaths occurred but slight decreases in body weight were seen during the study indicating that suitable doses were achieved. No adverse clinical signs were observed throughout the study.

In the negative control group there were 2 to 4 micronucleated cells (MNCPCEs) in 2000 polychromatic eryothrocytes per animal and the incidence of MNPCEs was 0.14%. The ratio of polychromatic erythrocytes to the total number of analysed erythrocytes (ratio of PCEs) was 57.1%. The incidences of MNCPEs after the administration of DHC were 0.14% in the 500 mg/kg group, 0.19% in the 1000 mg/kg group and 0.21% in the 2000 mg/kg group. No statistically significant increase was noted in any of the treatment groups compared with the negative control. These values were almost the same or smaller than the historical data from the testing facility (0.22%). The ratios of PCEs, an index of the test article on the bone marrow cells, were 58.9%, 59.2% and 61.6% in the 500, 1000 and 2000 mg/kg groups, respectively. No statistically significant difference was noted in any of the treatment groups compared with the negative control. In contrast, the incidence of MNCPEs in the positive-control group was markedly increased to 0.99% (11 to 25 MNCPEs in 2000 PCEs) which was statistically significant thus validating the sensitivity of the assay.

It was concluded that under the conditions of this *in vivo* assay in mice, DHC was non-clastogenic.

2.5 *In vivo* Comet assay in rats (Shimada S. 2007) Experiment No. 9993 (258-061) (Bernard BK et al., 2008a)

An in vivo Comet assay in rats was carried out in 2007 by the Biosafety Research Center (Shizuoka, Japan), 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, lot WKU05137ZBa, 95.8% pure) was diluted in medium chain triglyceride (MCT) that was also used as negative control. The positive control group received ethyl methanesulphonate (EMS) administered intraperitoneally.

Based on the outcome of the 13 week oral gavage rat study (Ref 2.1, Mochizuki M. 2006c) above, the test substance was administered daily by gavage (0.5 ml/100g BW) at the doses of 1000 and 2000 mg/kg (the maximum feasible dose) for 2 consecutive days. At the end of treatment, the intestinal tract, liver and kidney were selected, based on previous studies, as the potential organs

of absorption, metabolism and excretion for DHC. The tissues, taken 3 hours after the second dose, were homogenised in buffer, the cell suspensions chilled on ice, the supernatant discarded, the pellet removed and resuspended in a mixture of Hanks balanced salt solution, EDTA and DMSO and adjusted to pH 7.5 with NaOH. Three slides per organ were prepared and electrophoresis conducted.

No deaths occurred but a slight decrease in bodyweight was observed at the highest dose of 2000 mg/kg indicating that the maximum feasible dose also approximated to the maximum tolerated dose (MTD). No adverse clinical signs were seen.

One hundred cells per organ (50 cells per slide) ie 400 cells per group (4 animals) were examined. The percentage of DNA in the tail (%tail DNA) and Olive tail moment (Olive et al., 1990) were used as metrics.

Olive tail moment = (tail mean – head mean) x %tail DNA/100

Tail mean: centre of gravity of the tail
Head mean: centre of gravity of the head

The Olive tail moment and percent DNA were analysed statistically. Any DNA damaging potential was determined based on the presence or absence of significant differences in the Olive tail moment and percent tail DNA between the negative control group and treated dose group. The final judgement took into consideration the biological relevance under the test conditions and toxicity information on the test article.

In the liver, in the DHC group treated with 2000 mg/kg, the increase in the Olive tail moment and the mean % tail DNA were slightly increased (1.72 and 1.57 times that of negative control values respectively) being statistically significantly ($p < 0.05$ Dunnetts Test). However 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, 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 percent tail DNA were also slightly increased (1.35 and 1.21 times that of negative control, respectively) being statistically significant using Dunnett's test at $p = <0.05$). As can be seen there was no dose response. For the duodenum the mean values in the Olive tail moment and the percent tail DNA were only statistically significantly ($p < 0.05$) increased at 2000 mg/kg being 1.72 times and 1.45 times that of negative control values, respectively). Interestingly, 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 above the limit dose of 1000 mg/kg. 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.

Discussion and Conclusion on Toxicology of Laboratory Scale DHC

The findings in the 13 week study oral study were consistent with those using commercial grade DHC, the NOAEL was established to be 1000 mg/kg/day. The *in vitro* genotoxicity studies indicated positive findings for the Ames test and chromosome aberration test in the absence of a metabolising system (-S9) but not in the presence of S9. The *in vivo* clastogenicity study was negative but an equivocal within historical control result was found for DNA damage at the extremely high dose level of 2000 mg/kg. While equivocal, the weight of evidence suggests that this finding was fortuitous and unrelated to treatment, for the reasons discussed above.

3. Toxicology on CH-19 Sweet extract (Appendix 5)

The information comes from a range of toxicity and toxicokinetic studies conducted on CH-19 Sweet extract that contains approximately 7.5% of capsinoids (DHC, capsiate and nordihydrocapsiate). Of the capsinoids some 20% is DHC. Despite the relatively low levels of DHC per se, the remaining

capsinoids (capsiate and nordihydrocapsiate) are metabolised and excreted using a similar route and metabolite to that of DHC; thus exposure to capsinoids is complementary to the findings with DHC alone. The capsinoid toxicological database (Int J Toxicol, 27, Supplement 3, 2008) includes inter alia the results from an oral 2-generation reproductive toxicity study in rats which for the above reasons is deemed to complement and provide supportive information for DHC assessment.

The level of DHC, the compound under consideration, is identified for each study, see Table 17. All Lots conformed with specification and the individual Certificates of Analysis for each lot are presented in Appendix 1.

Table 17: Summary of Toxicity Studies Conducted on CH-19 Sweet extract (inc. DHC dose equivalence)

ID	Study Name	Lot No.	DHC dose equivalent mg/kg	Author (SD) Study/Exp't No.	Result mg/kg DHC
3.1	Single dose acute oral toxicity tests in rats	041029	71.25 142.50 285	Mochizuki M. 2005 B143	LD50 >285 mg/kg
3.2	13-week oral gavage toxicity study in rats	050331 M050419 050527	Low 16.63-20.19 Mid 33.25-40.38 High 66.50-80.75	Mochizuki M 2006a B180	NOAEL 66.5 to 80.75*
3.3	26-week oral gavage toxicity study in rats	050331 M050419 050527 050530	Low 16.63-20.19 Mid 33.25-40.38 High 66.50-80.75	Mochizuki M 2006b N-B145	NOAEL in Males 33.25 to 40.38* NOAEL in Females 66.5 to 80.75*
3.4	Oral gavage teratology and developmental toxicity study in rats	050530	Low 20.19 Mid 40.38 High 80.75	Katsumata Y 2006a N-R013	Maternal and Fœtal NOAEL 80.75
3.5	Oral gavage developmental toxicity study in rabbits	050404	Low 3.8 Mid 7.6 High 15.2	Matsuoka T. 2006 N-R010	Maternal and Fœtal NOAEL 15.2
3.6	Two-generation oral gavage reproduction study in rats	050318 050331 M050419 050404 050527 050530 050531	14.25 - 20.19, 28.5 - 40.38 57 - 80.75	Katsumata Y 2006b N-R008	NOAEL 57 to 80.75*
3.7	Bacterial reverse mutation test	050331	-	Nakajima M. 2005 9224 (258-041)	Not mutagenic
3.8	*In vitro* chromosome aberration test	050331	-	Masumori S. 2005a 9225 (258-042)	Not clastogenic
3.9	Mouse micronucleus test	050331	-	Masumori S. 2005b 9226 (258-043)	Not clastogenic

ID – Identification in Section
SD- Study Director/Author of Original Study Report
*due to range of DHC content in different Lots of CH-19 Sweet extract
- plate concentration conversion in DHC equivalent not calculated

3.1 Single dose acute oral toxicity test in rats (Mochizuki M., 2005) Study No. N-B143 (Watanabe et at., 2008c)

An acute oral toxicity study was conducted in rats by Bozo Research Centre Inc in 2005.

Sprague-Dawley rats, (5/sex/group) were administered CH-19 Sweet extract (71.25 mg/ml capsinoids) by single oral gavage at dose levels of 0 (medium chain triglyceride vehicle control), 5, 10 and 20 ml/kg (the maximum dose of capsinoids was 1425 mg/kg which containing 20% DHC equates approximately to doses of 71.25, 142.50, 285 mg DHC /kg). No deaths occurred. Salivation was seen in females from the 2 highest dose groups and decreased spontaneous activity in both sexes thereafter. These signs were absent 4 hours after dosing. Soft faeces were interpreted to be due to the large amount of the oily vehicle. There were no significant differences in bodyweight and no abnormal findings at macroscopic post-mortem examination.

It was concluded in the absence of any mortalities that the LD50 was >20 ml/kg CH-19 Sweet extract ie approximately 285 mg/kg DHC.

3.2 13-week oral gavage toxicity study in rats (Mochizuki M., 2006a) Study No. N-B180

A 13-week subchronic oral gavage study of CH-19 Sweet extract was performed in 2006, by the Bozo Research Center Inc.

The study was carried out in Sprague-Dawley rats, employing 10/sex/group, dosed for 13 weeks at levels of 0, 1.25, 2.5, 5.0 ml/kg/day (approximately equivalent to 16.63 to 20.19, 33.25 to 40.38, and 66.5 to 80.75 mg DHC/kg/day according to the Lot employed). The total capsinoid content varied in the range between Lot M050419 (356.25 mg/kg) and and Lot 050331 (365.75 mg/kg) capsinoids at the high dose level according to the Lot employed and the DHC between 13.3 and 16.15 mg/ml, respectively.

There were no treatment related sign in the low or mid-dose groups whereas the high-dose rats showed occasional salivation. While this was seen in all animals, the incidence each day was only 2 or 3 animals/sex/day. Bodyweight, food and water intake were unaffected by treatment with the test article as was the ophthalmology and haematology. A statistically significant reduction in MCV and MCH observed in high dose females was considered to be of doubtful toxicological significance as the findings were minimal (lower than control by ≤4%), not associated with any changes red blood cell count, haematocrit, or haemoglobin concentration: moreover no abnormalities were seen in the hematopoietic organs on histopathological examination. Mid- and high-dose females demonstrated a small but significant prolongation in prothrombin time (mid-dose 13.92 ± 0.4 seconds, range 12.9 - 14.5 seconds; high-dose 14.0 ± 0.5 seconds, range 13.2 - 14.6 sec). These findings were interpreted as not being treatment related as they were only slightly higher than the range for the control group (12.6 - 14.1 sec). No changes in any other coagulation parameters were observed and no evidence of hemorrhage was observed during the histopathological examination. At first evaluation there appeared to be an increase in the AST, ALT and LDH values in high-dose males. However on further inspection it was noted that this resulted from an increase in male rat No. 4002 (AST 391 IU/L, ALT 358 IU/L and LDH 226 IU/L; histopathological examination of this animal revealed focal necrosis and bile duct proliferation in the liver. However no other significant blood biochemical or histomorphological changes in the liver were seen of any of the other animals. A slight statistically significant increase in absolute and relative (11%) liver weight was observed in the high-dose male group. No response was observed in the females. In the light of only one male animal (4002) showing an hepatic lesion, with no histopathological or blood biochemical changes indicative of hepatotoxicity in any of the other males or females and only a slight increase in liver weight in high males probably reflecting a physiological adaptation to work-load, the effect in the isolated male was considered to be incidental to treatment with CH-19 Sweet extract.

However, small differences in the incidence and degree of focal myocarditis were observed in males in each test group, compared with control.

Sex	Males				Females			
Dose level (ml/kg/day)	0	1.25	2.5	5.0	0	1.25	2.5	5.0
Number of animals	10	10	10	10	10	10	10	10
Heart Focal Myocarditis								
±	2	5	5	4	2	/	/	4
+	0	3	4	5	0	/	/	0

± minimal
\+ mild

In consequence a Pathology Working Group (PWG) was convened and reviewed the histopathological findings of focal myocarditis. The PWG concluded there is no evidence that the myocarditis was chemically-induced and that the incidence and severity of the lesions were comparable to those expected from spontaneous cardiomyopathy. (See Section 3.3 below for full details and the composition of the PWG).

Based on these findings, the NOAEL in males and females was considered to 5.0 ml/kg/day CH-19 Sweet extract approximately equivalent to 66.5 to 80.75 mg/kg/day DHC.

3.3 26-week oral gavage toxicity study in rats (Mochizuki M., 2006b) Study No. N-B145 (Kodama, et al 2008b)

A 26-week subchronic oral gavage study of CH-19 Sweet extract was performed in 2006, by the Bozo Research Center Inc., Japan.

Twenty male and 20 female Sprague-Dawley rats/group were dosed orally by gavage at 0, 1.25, 2.5, 5.0 ml/kg (approximately equivalent of 16.63 to 20.19, 33.25 to 40.38, and 66.5 to 80.75 mg/kg DHC (according to the Lot used). The total capsinoid content varied in the range between Lot M050419 (356.25 mg/kg) and and Lot 050331 (365.75 mg/kg) capsinoids at the high dose level according to the batch employed and the DHC between 13.3 and 16.15 mg/ml, respectively.

Occasional signs of salivation were observed in high-dose level animals from day 15 of administration onward, with an incidence of 3 to 7 rats of either sex. There were no effects on bodyweight, food or water intake.

Significantly high values were observed in the percentage of segmented neutrophils in high-dose males and significantly low values in the percentage of eosinophils in high-dose females. The low value of eosinophils was determined not to be treatment related as the individual percentage and individual absolute number were within the range of the control group. A low value in the percentage of lymphocytes was observed in high-dose males; however it was not different in the absolute number as compared to controls. All treated males demonstrated significant decreases in PT and APTT. These findings were determined not to be toxic since the results reflect shortening of PT and APTT and not prolongations. There was no associated pathological change and the decrease in APTT was not dose-related.

Although significant increases in the urinary excretion of sodium was observed in mid- and high-dose males and high-dose females and potassium in high-dose females, there were no changes in associated blood chemistry values. Thus findings were determined not to be treatment related. Females in the high-dose group demonstrated a significant increase of chloride in urine and a decrease of chloride in blood chemistry; the elevated level of chloride was determined not to be treatment related due to the lack of other abnormal renal function parameters and the lack of abnormalities upon histopathological examination.

Significantly higher group mean values were observed in AST, ALT and LDH in high-dose males although this was predominatly due to 5 animals when comparing against control, ie one quarter of the high-dose male group. There was also a slight increase in liver weight when corrected for

bodyweight of approximately 16% in this group. Minimal focal liver necrosis was observed in one male in the control, low and mid-dose groups and in 3 males in the high-dose group, a fourth high-dose animal showed moderate focal necrosis. Three of these high dose animals also had increased liver enzyme values. Taken together there is evidence for possible treatment related liver toxicity (with a very slight increase compared with the other treated groups and control ie 4 affected v. 1) in a small proportion of male high-dose level rats but not in females.

Myocardial fibrosis was observed in 14 males and 5 females in the control group, 13 males and 8 females in the high-dose group. Focal myocarditis was observed in 18 males and 15 females in the control group, and 17 males and 14 females in the high-dose group. A Pathology Working Group (PWG) reviewed these histopathological findings and concluded there is no evidence the myocarditis was chemically-induced and the incidence and severity of lesions demonstrated were comparable to those expected from spontaneous cardiomyopathy. (See below for a discussion of the PWG process and findings)

Based on these findings, the NOAEL was considered to to be 5.0 ml/kg CH-19 Sweet extract in females and 2.5ml/kg in males, approximating to 66.5 to 80.75 and 33.25 to 40.38 mg DHC/kg.

Background to and Composition of Pathology Working Group

On October 2nd, 2006 the FDA returned comments on a New Dietary Ingredient Notification submitted by Ajinomoto. The FDA indicated that it had concerns about the evidence presented to support the conclusion that a dietary supplement containing "Capsinoids will reasonably be expected to be safe." The FDA communication specifically commented on results from the 13-week oral toxicity study of CH-19 Sweet extract (Study Number N-B180). The FDA stated that "the information provided from this study raises concerns about cardiac toxicity of 'Capsinoids (Extracted Oil of Sweet Chilli Peppers)' and was inadequate to allow FDA to evaluate the basis for the safety of your product. For example, there were highly significant, treatment-related increases in the incidence and degree of focal myocarditis in male rats in each test substance group." Based on the situation above, a Pathology Working Group (PWG) was established and reviewed heart sections independently from the applicant. The findings of this PWG were submitted to FDA by the applicant, and after reviewing its findings, the FDA withdrew its objections and accepted the dietary ingredient notification for their file on May 17, 2007.

Studies Reviewed

A Pathology Working Group (PWG) Review was performed to review the heart sections from two oral toxicity studies and a reproduction study conducted in rats with CH-19 Sweet extract. These studies are listed as follows:

- A 13-Week Oral Toxicity Study of CH-19 Sweet extract in Rats (Study Number N-B180)
- A 26-Week Oral Toxicity Study of CH-19 Sweet extract in Rats (Study Number N-B145)
- A Reproduction Study of CH-19 Sweet extract By Oral Administration in Rats (Study Number N-R008)

PWG Charge

The purpose of this review was to have a panel of pathology experts examine heart sections to evaluate the findings reported by the Study Pathologist and to provide a consensus diagnosis for the heart for each animal evaluated. In addition, the PWG provided its consensus diagnoses for heart sections of groups of male rats in the 13-week study and male and female rats in the 26-week study that were not evaluated by the Study Pathologist for each study. The PWG specifically focused on the presence or absence of heart microscopic morphologic changes that might be consistent with myocarditis, myocardial fibrosis or the continuum of degenerative and inflammatory myocardial changes commonly referred to as cardiomyopathy. The PWG review

was conducted using procedures similar to those followed routinely by the pharmaceutical companies and regulatory authorities. The PWG panel was also requested to provide a discussion on the toxicological significance of the microscopic changes in the heart of rats orally dosed with CH-19 Sweet extract.

Members of the PWG

The Pathology Working Group was chaired by Dr. Henry Wall, Experimental Pathology Laboratories, Inc. (EPL®), who organized and presented the material to the panel of five pathologists with specific expertise in the cardiovascular system and/or with regulatory toxicologic pathology. The PWG Chairperson and all experts selected as PWG Participants were veterinary pathologists certified by the American College of Veterinary Pathologists. Individuals participating in the PWG or attending as an observer are listed as follows:

Dr. Henry G. Wall	Chairperson
Dr. W. Ray Brown	PWG Participant
Dr. Charles B. Clifford	PWG Participant
Dr. Jerry F. Hardisty	PWG Participant
Dr. Ernest E. McConnell	PWG Participant
Dr. Paul W. Snyder	PWG Participant
Dr. George Burdock	Observer
Dr. Yoshiyuki Fujishima	Observer

PWG Method and Discussion

The PWG was provided with all the heart section microscopic slides from the 3 studies (both male and female). The PWG examined coded slides without knowledge of treatment group and previous diagnosis. The animals were randomized using a computerized random number generator before they were coded. After an initial round of review of hearts from 20 rats with changes representing the spectrum of diagnoses made by the Study Pathologist, the PWG discussed terminology and concluded that myocarditis and myocardial fibrosis were part of the continuum of changes that is commonly referred to as cardiomyopathy (Jokinen et al., 2005; MacKenzie and Alison, 1990).

The PWG also considered that the use of a single term would also more accurately reflect incidence of lesions that represented a continuum of related morphologic effects. The PWG findings for each animal were discussed by the group, re-examined if necessary to assure that all pertinent structural features bearing on the diagnosis were considered by all PWG panel members, and the final opinions were recorded on the Chairperson's worksheets. The consensus diagnoses of the PWG were reached when at least three of the five PWG participants were in agreement.

PWG Conclusions

The PWG concluded that:

1. The myocardial lesions observed in animals that received CH-19 Sweet extract are consistent in structural character, distribution, and severity with myocardial lesions associated with spontaneous cardiomyopathy, an age-related progressive condition that is common to the Sprague-Dawley, Wistar and Fischer 344 rat strains (Jokinen, et al., 2005; Kemi et al., 2000; Lewis, 1992; Ruben et al., 2000).

2. The differences in incidence and severity with dose and duration of dosing as evidenced in the three studies do not provide evidence of a pattern of widespread myocardial injury as would be expected for chemically-induced myocarditis.

3. The myocardial atterations observed in rats orally administered CH-19 Sweet extract in these studies are not toxicologically significant since the incidence and severity of the lesions are comparable to those expected for spontaneous cardiomyopathy.

3.4 Oral gavage teratology and developmental toxicity study in rats (Katsumata, Y., 2006a) Study No. N-R013 (Bernard BK et al., 2008c)

An oral gavage teratology study in rats was performed on CH-19 Sweet extract in 2006 by the Bozo Research Center Inc, Japan. The study 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).

Twenty pregnant Sprague-Dawley dams/group were dosed orally by gavage for 11 consecutive days, from day 7 to 17 of gestation at levels of 0, 1.25, 2.5, 5.0 ml/kg CH-19 Sweet extract (Lot 050530) (equivalent to 20.19, 40.38 and 80.75 mg DHC/kg). The vehicle alone, MCT, was given to the controls.

There were no deaths, nor dams with premature delivery/abortion in any groups. The only abnormality observed during gestation was salivation in two females in the high-dose group on Day 17 of gestation. The mid-dose group demonstrated a significant increase in body weight gain on Day 18 to Day 20 of gestation. The low and mid-dose groups had significant increases in food consumption from Day 14 to Day 18 of gestation as compared to controls. Food consumption tended to be higher in the high-dose group as compared to controls during this period. These findings were only observed during Days 14-18 of gestation. Gross pathological examination of the thoracic and abdominal cavities of the dams following caesarian section revealed no evidence of any treatment related findings in any group.

No significant differences were observed in the number of corpora lutea, number of implantations, implantation index, index of dead embryos/foetuses, or number of live foetuses. No significant differences in sex ratio or body weight of live foetuses were observed in any group. For external abnormalities, short trunk with vestigial tail was observed in one foetus in the high-dose group. There were no macroscopic abnormalities in the placentas of foetuses in any group and placental weights were comparable across all groups. Visceral abnormalities and variations were observed in foetuses from all groups; however, all incidences were comparable between controls and test groups. For visceral abnormalities the figures were comparable between the historical control data in the testing facility (3.1% ± 7.1%) and the study control data (3.8% ± 8.0%) and the treated groups low (0.6% ± 2.8%), mid (5.4% ± 7.7%) and high-dose (1.5% ± 4.5%). The abnormalities observed were dilatation of lateral ventricle, abnormal origin of the left pulmonary artery, ventricular septal defect, and abnormal lobulation in the liver. The variations observed were thymic remnant in the neck, dilatation of renal pelvis and dilatation and convolution of the ureter. As with the visceral abnormalities, the incidences of theses variations were comparable between the historical control data in the testing facility (7.1% ± 11.2%) and the control data in this study (5.7 ± 9.7%), and also between the control group and each test substance administration group. Results of skeletal examination showed a skeletal abnormality, a wavy rib, in 1 foetus in the low-dose group. Although skeletal variations were observed in all groups, there were no significant differences in incidences between control and treated groups. As for ossification, there were no significant differences between the control group and any treated group in the index of ossified sternebrae, or the number of ossified metacarpi, metatarsi, ossified sacrococcygeal vertebrae.

The study concluded the NOAEL for this study is 5.0 mL/kg/day CH-19 Sweet extract (80.75 mg/kg/day DHC). (Bernard, et al., 2008d)

3.5 Oral gavage developmental toxicity study in rabbits (Matsuoka, T., 2006) Study No. N-R010 (Bernard BK., et al 2008c)

An oral gavage teratology study in rabbits was performed on CH-19 Sweet extract in 2006 by the Bozo Research Center Inc, Japan. The study 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).

Seventeen to 22 pregnant female New Zealand white rabbits/group, were dosed orally by gavage from day 6 to day 18 of gestation, at dose levels of 0, 0.25, 0.5, and 1 ml/kg CH-19 Sweet extract Lot No. 050404 (approximately equivalent to 3.8, 7.6, 15.2 mg DHC /kg). The controls receieved the MCT vehicle alone.

The number of non-pregnant rabbits was 2-7/group. There were no deaths in any of the test groups. Abortion was observed in one female in the low-dose group. A decrease in faeces, similar to that of the control group, was exhibited in all test groups. There were no significant differences in group mean body weight or group mean body weight gain. No statistically significant differences in group mean food consumption were noted. Gross pathology revealed no abnormalities in any of the four groups or in the aborted fetus. There were no significant differences in treated groups as compared to controls in the number of corpora lutea, number or implantations or index, or number or index of embryo-foetal deaths.

Following caesarian section, no significant differences were observed in the number of male or female live foetuses, sex ratio, or body weight of male or female fetuses. No gross pathological abnormalities in the placenta and no changes in placental weight were observed. Gross pathological examination for external abnormalities showed meningocele and syndactyly in the forelimb, each in one fetus in the control group, cleft of abdominal wall in one foetus of the mid-dose group and club foot in one foetus of the high-dose group. These external abnormalities did not represent a significant difference in incidence of treated groups as compared to controls.

Visceral abnormalities and variations and skeletal abnormalities and variations were observed in all groups. However, there was no significant difference in the number of foetuses with these changes between controls and treated groups. The number of live foetuses with skeletal abnormalities was 5 in 4 litters (3.6% ± 7.8%) in the control group, 1 in 1 litter (0.5% ± 2.4%) in the low-dose group, 1 in 1 litter (0.7% ± 3.1%) in the mid-dose group and 4 in 4 litters (3% ± 5.6%) in the high-dose group. Regarding the progress of skeletal ossification, there were no significant differences between the control group and any of the treated groups.

It was concluded that the NOAEL for this study (dams and foetuses) was the high dose level of CH-19 Sweet extract approximately equivalent to 15.2 mg/kg DHC.

3.6 Two-generation oral gavage reproduction study in rats (Katsumata, Y., 2006b) Study No. N-R008 (Kodama T., et al 2008c)

An oral gavage 2-generation study in rats was performed on CH-19 Sweet extract in 2006 by the Bozo Research Center Inc, Japan. The study 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).

Twenty-four Sprague-Dawley rats/sex/group were employed in the parental F_0 generation, and treated at doses levels of 0, 1.25, 2.5, 5.0 ml CH-19 Sweet extract/kg (equivalent to 14.25 to 20.19, 28.5 to 40.38, and 57 to 80.75 mg DHC/kg according to the Lot employed). Controls received the medium chain triglyceride vehicle alone. Administration was started 8 weeks before the start of mating for F_0 males, 2 weeks before the start of mating for F_0 females, throuhout pregnancy and up to weaning for F_1 rats (day 21, after birth). For both F_0 and F_1 rats, administration was continued until the day before necropsy for males and to day 20 of lactation for females.

F_0 Generation

In summary, there were no test article-related deaths and no findings of toxicological significance in clinical signs, body weight, food consumption, or gross pathology. There were no treatment related effects on the number of oestruses, oestrous cycles, copulation index, the number of days before copulation, fertility index, number of implantations, gestation period, number of live pups, delivery index, still birth or live birth index, or in nursing behaviour.

Transient salivation following dosing was observed in all groups including the controls, this was slightly more marked in the high-dose males and females. This effect was not considered to be of toxicological significance. One male in the high-dose group died from aspiration of ingesta which was classified as a non-test article related accidental death. No statistically significant differences in body weights of males and females compared with controls were observed except in mid-dose females (slightly lower weights) during the pre-mating period. In the low-dose group, increased food consumption was observed in males from Day 36 onward, compared to controls. In terms of reproductive indices, the stillbirth index was significantly lower in the lowdose group and the number of implantations and stillbirth index were significantly lower in the mid-dose group compared with controls. There were no significant differences in the number of implantations and still birth index in the high-dose group compared with controls. At necropsy one male in the mid-dose group was observed to have a focal adhesion of the lung to the thoracic wall and opacity of the pericardium. In the control group, one male presented with bilaterally small testes and epididiymes. None of these findings were indicative of treatment related effects.

F_1 Generation

There were no test article related effects on sex ratio at birth or body weights of live pups, and no external abnormalities. Similarly there were no significant differences in the viability index on day 4 or the weaning index on day 21 after birth. The low and high-dose groups demonstrated a significantly lower index of eyelid opening on Day 14 after birth. This was determined not to be treatment-related since eyelid opening was observed in all animals by Day 17 and there was nothing suggestive of growth retardation (ie., changes in body weight). The incisor eruption index on Day 11 after birth was significantly higher in the low and mid-dose groups. No significant differences were observed in the index of pinna detachment, opening of vagina or cleavage of the balanopreputial gland between controls and any test group. Necropsy of pups that died revealed no test article-related gross abnormalities of the main organs in the thoracic and abdominal cavities. Two males and one female in the control group, one male and three females in the mid-dose group and one female in the high-dose group presented with thymic remnant in the neck on Day 4 after birth. There were no other organ abnormalities in the thoracic or abdominal cavity; this finding was considered to be incidental. One male in the low-dose group at weaning was found to have thymic remnant in the neck. Necropsy of stillborn pups revealed bilateral ureteral dilatation in one female in the high-dose group. No other macroscopic abnormalities were observed in the main organs or tissues of the thoracic or abdominal cavities; this finding was determined to be unrelated to treatment.

In the sensory/reflex tests Preyer's reflex was normal in all groups. Mid-dose males and females demonstrated significantly higher air righting reflex index compared with controls. Reaction time of righting reflex was not significantly different in any of the groups. The control, the low and mid-dose groups showed a prone position post-dosing beginning at one and two weeks of treatment. This was considered to be the effect of dosing the vehicle to very young (3-4 week old) rats as the frequency was highest in the controls. Similar to the F_0 generation, sporadic increases in salivation were observed in males and females in the mid and high-dose groups. High-dose males had significantly higher body weight as compared to controls from Day 56 after birth and on. As compared to controls, low-dose males body weight from Day 133 after birth onward. The mid-dose male body weight was not significantly different from controls. No significant differences were demonstrated in female body weight or body weight gain from the day of birth to the starting day of mating, during gestatign period or during lactation in any of the groups. No significant differences were demonstrated in the number of oestruses, oestrous cycle, copulation index, the number of days until copulation, fertility index, station period, number of live pups, delivery index, stillbirth or live birth index in any of the groups. One dam in the mid-dose group had insufficient lactation on Day 2 that resulted in a complete loss of the pups. This finding was observed in only this one animal and no similar abnormalities were found in any other treatment groups. One male in each ofthe control, mid and high-dose group showed a dark red area of the liver. An enlarged thymus was observed in one mid-dose male. One female in the low-dose group that died presented a dark area in the lung and an enlarged spleen, liver, and adrenal glands. No macroscopic abnormalities in other organs in the thoracic or abdominal cavity in any other tested animal. Focal myocarditis was found in 15, 17, 18, 17 males and myocardial fibrosis in 2, 4, 2, and 5 males in the control, low-, mid- and high-dose, respectively. These findings were

addressed by the Pathology Working Group; the PWG concluded that there was no evidence that these findings were test article related. (See Sub-Section 3.3 above for a discussion of the WG process and findings)

F_2 Generation

There were no significant differences in sex ratio at birth or in body-weight and no external abnormalities. The high-dose group demonstrated a significantly higher index of eyelid opening on Day 14 after birth, as well as air righting reflex as compared to controls. As with the previous generation, there was no evidence of growth disturbance based on body-weight. No significant differences were observed in any of the groups with regard to pinna detachment or incisor eruption, the reaction time of the righting reflex or Preyer's reflex. No macroscopic abnormalities of the main organs and tissues of the thoracic or abdominal cavities were observed. On Day 4 after birth, gross pathology examination revealed one female in the low-dose group with opacity of the right median lobe of the liver, and thymic remnant in the neck in two females in the high-dose group. No other abnormalities were observed. During gross pathological examination following weaning, microphthalmia was observed in one male and one female in the low-dose group. One female in this group demonstrated caecal obstruction. No other macroscopic abnormalities were observed.

The report concluded that the NOAEL for CH-19 Sweet extract was 5.0 ml/kg (the equivalent of 57 to 80.75 mg/kg DHC).

3.7 Bacterial reverse mutation test (Nakajima, M., 2005) Experiment No. 9224 (258-041) (Watanabe, et al., 2008c)

A bacterial reverse mutation test, with and without metabolic activation was conducted by the Biosafety Research Centre, Foods, Drugs and Pesticides, An-Pyo Center, Shizuoka, Japan. The study was conducted referring to “The ordinance on standards for conduct of non-clinical studies on safety of drugs”, Ordinance No. 21 of the Ministry of Health and Welfare, Japan (March 26, 1997).

The assays were carried out in *Salmonella typhimurium* strains TA 100, TA98, TA1535 and TA1537 and *Escherichia coli WP2uvrA*.

An initial dose-ranging study was conducted and the highest dose of 5000 µg/plate was established to cause bacterial growth inhibition. Precipitation was also seen in the -S9 mix from 625 µg/plate and from 1250 µg/plate with the +S9. A dose range up to 5000 µg/plate CH-19 Sweet extract (Lot No. 050331, having a capsinoid content of 73.15 mg/ml), the maximum practicable dose level, was employed in the definitive test. While the different tester strains showed different tolerance/growth inhibition to the dosages employed there was no increase in revertant colonies either in the presence or absence of metabolic activation in any of the strains up to the maximum tolerated dose.

Based on these results, it was concluded that CH-19 Sweet extract does not induce gene mutation.

3.8 In vitro chromosome aberration test (Masumori, S 2005a) Experiment No. 9225 (258-042) (Watanabe, et al., 2008c)

A chromosome aberration test, with and without metabolic activation (S9), was conducted by Biosafety Research Centre, Foods, Drugs and Pesticides, An-Pyo Center, Shizuoka, Japan. The study was conducted referring to “The ordinance on standard for conduct of non-clinical studies on safety of drugs”, Ordinance No. 21 of the Ministry of Health and Welfare, Japan (March 26, 1997).

Dosage to chinese hamster lung fibroblast cell line (CHL/IU), was based on a preliminary cell growth inhibition study. Short term, with and without S9 mix, and continuous 24-hr treatment was undertaken, the former using a dose range of 2450-5000 µg/ml and the latter a dose range from 1201 to 2450 µg/ml. No chromosome aberrations were observed either in the presence or absence of metabolic activation. The positive controls mitomycin C and cyclophosphamide

(short term treatment) and mitomycin C (continuous treatment) induced a high incidence of aberrations.

It was concluded that CH-19 Sweet extract did not induce chromosomal aberrations in cultured mammalian cells under the conditions of this study.

3.9 Mouse Micronucleus test (Masumori, S 2005b) Experiment No. 9226 (258-043) (Watanabe et al., 2008c)

An oral gavage micronucleus test was conducted by Biosafety Research Centre, Foods, Drugs and Pesticides, An-Pyo Center, Shizuoka, Japan in male mice (BDF_1 strain), 5 mice/group, with daily dosing for two consecutive days. The study was conducted referring to "The ordinance on standard for conduct of non-clinical studies on safety of drugs", Ordinance No. 21 of the Ministry of Health and Welfare, Japan (March 26, 1997).

A positive control of mitomycin C, (MMC via ip administration at 2ml/kg), a negative control, and the extract were administered at 5, 10, 20 ml/kg the high-dose level representing the maximum practicable gavage dose.

No statistically significant increase in the incidence of micronucleated polychromatic erythrocytes (MNCPE) was observed in any group compared with control. Moreover there was no decrease in the ratio of polychromatic erythrocytes to the total number of analysed erythrocytes in any of the treatment groups. In contrast, the incidence of MNCPE in the positive control group was markedly and statistically significantly increased.

It was therefore concluded that CH-19 Sweet extract was not clastogenic under the conditions of this study.

Discussion and Conclusion on Toxicology of CH-19 Sweet extract

The toxicological profile of CH-19 Sweet extract at the limit dose was found to be generally consistent with that of DHC albeit that the DHC content was considerably lower, (than when dosed as pure DHC at 1000 mg/kg). There was no evidence of teratogenicity, genotoxicity or adverse effects on fertility or general reproductive performance. The NOAEL for all of the repeat dose studies was the highest dose that could be administered using the CH-19 Sweet extract with the exception of males treated for 26 weeks. Here a possible treatment related response (liver enzyme and organ weight increase with associated focal necrosis) was seen in approximately 25% of the high dose male rats.

XIII.2.4 Human Safety Assessment Studies on DHC (Appendix 3)

1. Commercial Grade DHC (Appendix 3)

8-day capsule repeat dose safety assessment study in healthy male volunteers Study No. AMO-08-03 (Kajimoto Y. 2009)

The safety of 8 days repeated oral ingestion of DHC, Capsule Lot No. 19FZY2 containing DHC (Lot No. 070813) was evaluated in 34 healthy, fasted Japanese male volunteers (average age 50.8yrs), using a randomized, placebo-controlled, double-blind, parallel groups study. Subjects received either three or twelve, 1 mg capsules, representing a dose of 3 or 12 mg of DHC, respectively. The study was conducted in the Soiken Clinic, Japan.

The dosing with DHC well-tolerated and none of the subjects discontinued dosing due to subjective signs/symptoms or adverse events.

The overall incidence of subjective symptoms was similar in the placebo (3/11), DHC low-dose group (2/12), and high-dose group (1/12). Signs were recorded in 5 subjects in total with 1 volunteer showing raised cholesterol and BUN. The reported findings varied from subject to subject and included fatigue, cough, and sore throat (n=1, respectively) in the placebo group, stiff shoulders (n=1), high total cholesterol and blood urea nitrogen (n=1) in the high-dose group, and constipation, bradycardia and reduced blood pressure (all events occurred in the same subject) in a single low-dose volunteer. Generally there were no dose-related trends or patterns to

indicate a treatment relationship. Signs assessed as unclear in aetiology and possibly related to DHC were stiff neck, high total cholesterol and blood urea nitrogen (BUN) in the high-dose DHC group, and constipation, bradycardia and reduced blood pressure in one volunteer in the low-dose group. There were no clinically meaningful differences observed between the placebo and DHC groups with regard to changes from baseline for clinical laboratory parameters, since the variations were within the normal range and there was no evidence of any dose-relationship.

A small but statistically significant increase of diastolic blood pressure was observed in the high-dose group on Day 8 compared with baseline (pre-treatment), but this increase was thought not to be clinically significant since the baseline diastolic blood pressure on Day 8 was lower than that before dosing commenced on Day 0.

In conclusion DHC was well tolerated in an 8-day oral repeat dose capsule study at dosages up to 12 mg/day, equivalent to 0.2 mg/kg bw/day for a 60 kg human. There were no consistent trends in signs or symptoms between the volunteers at the same dosage levels and no abnormalities in the haematology or clinical chemistry, blood pressure or heart rate.

1.1 4-week beverage repeat dose safety assessment study in healthy male volunteers Study No. AMP-2008-06 ((Kajimoto Y. 2009)

The safety of repeated oral ingestion of DHC Lot No. 060712 was evaluated in 70 healthy, Japanese male volunteers (average 43.8 yrs), using a randomized, placebo-controlled, double-blind, parallel groups study. Subjects were randomized for treatment and received 3 or 9 mg equivalent of DHC as an ingredient in the beverage or the placebo treatment without having any active ingredients. The treatment duration was 4 weeks and the study was conducted in the Soiken Clinic, Japan.

DHC was safe and 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 (4/24) and DHC low-dose group (2/23) and high-dose group (3/23). The most common findings were headache (1 volunteer ie 4.2% in the placebo group and 2 volunteers, 8.7% in the high-dose DHC group) and rhinitis (4.2% and 4.3%, respectively). Other signs included heartburn (high-dose, 4.3%), sore throat (low-dose, 4.3%), loose stool (low-dose, 4.3%), dermatitis (placebo, 4.2%), atopic dermatitis (placebo, 4.2%) and a decrease of body weight (placebo, 4.2%). Only the loose stools in the low-dose DHC group (seen only in 1 volunteer) was assessed to be possibly related to the study material. This finding together with an increased frequency of defaecation appeared after the start of consumption of the test food and subsided 2-3 days after its completion. However, this was considered to be of doubtful clinical significance because the same sign was not observed in the high-dose group. All of the other reported signs were considered to be incidental as there was no pattern, trend or dose relationship.

There were statistically significant changes between Day 0 (pre-treatment, baseline) and Day 28 (post-treatment) in several blood chemistry parameters in all groups, and there were statistically significant differences observed between the placebo and DHC groups with regard to changes from baseline on Day 0. However, the variations were assessed to be of no clinical significance since the changes were within the normal range of biological variation and no significant differences were observed between the placebo and DHC group on Day 28.

A statistically significant increase of systolic blood pressure from baseline (pre-treatment) was observed in the high-dose DHC group 40 minutes after taking the study material on Day 0 and the diastolic pressure was higher in the low and high-dose groups, compared with placebo, at this time as well. This finding was thought to be of doubtful clinical significance, since the increase was transient on Day 0 and was not observed on Day 28. No subject showed any clinically significant increase in the systolic or diastolic blood pressure based on the results of a review of individual data for each subject.

In conclusion 3 mg or 9 mg volunteer/day administered in a beverage over 4 weeks was found to be well tolerated by a large group of volunteers. The dosages equated to approximately 0.05 mg/kg bw/day and 0.15 mg/kg bw/day based on a bodyweight of 60kg. The only finding that could be potentially related to treatment was the presence of loose faeces in 1 low dose-level

volunteer. As there was no evidence for this at the high dose level, the finding was to considered to be of doubtful biological significance.

Conclusion on Human Safety and Tolerance to DHC

DHC, administered in capsules at 3 or 12 mg volunteer/day for 8 days or at 3 or 9 mg volunteer/day for 4 weeks in a beverage, was well tolerated and gave rise to no obvious dose related clinical signs or other treatment related effects. The occasional and sporadic findings recorded in the 2 separate studies seldom occurred in more than 1 volunteer/sign and there was no consistent pattern or trend observed. Analysis of the individual data in an attempt to determine any relationship with treatment showed no clear changes. Minor variations in blood pressure appeared unlikely to be related to treatment based on increases in one study and reductions in the other.

2. CH-19 Sweet extract (Appendix 5)

2.1 Single dose capsule safety assessment study in healthy male volunteers (Hamada M., 2007) Study No. F0603 (Bernard et al., 2008d)

This study was conducted at the Kurume Clinical Pharmacology Clinic, Japan in accordance with the ethical principles of set forth in the Declaration of of Helsinki. The study was conducted at and approved by the Institutinal Revew Board of Kurume Clinical Pharmacology Clinic, Japan.

The safety and pharmacokinetics of a single oral dose of CH-19 Sweet extract was evaluated in 24 healthy, fasted Japanese male volunteers (20-38 yrs, 161.6 to 187.5 cm for height and 57.0 to 80.8 kg for weight), using a randomized, placebo-controlled, double-blind, parallel groups study design. Subjects received either 3 or 6 capsules, each containing approximately 5 mg CH-19 Sweet extract Capsule Lot No. 18F113, resulting in 15 or 30 mg of CH-19 Sweet extract/subject, respectively (equivalent to 3.96 mg and 7.92 mg of DHC, respectively). Placebo subjects of both groups received 6 placebo capsules only. Capsules were taken at a single point in time (in the morning), in a fasting state and with 50 ml water.

All subjects were monitored for safety and tolerance to the test article using physical examinations, subjective assessment/adverse events reporting, body weight, laboratory tests (hematology, blood chemistry, and urinalysis), vital signs (blood pressure and heart rate), ECG and body temperature, plasma concentrations of capsinoids and vanillyl alcohol, and plasma concentrations and urinary excretion of catecholamines and their metabolites. Most of these measurements were taken before dosing, and at 0.25, 0.5, 1, 2, 3, 4, 8, 24hours and 7 days post-dosing, (the results of the PK study are given in Sub-Section XIII.2.2).

CH-19 Sweet extract was well-tolerated, and no clinically significant effects were observed even when 30 mg of capsinoids (approximately equivalent to 7.92 mg DHC) was ingested. Three subjects experienced either mild headache (one low-dose subject), mild aphthous stomatitis (one high-dose subject), or mild pharyngeal pain (one high-dose subject), after ingestion of the capsules of CH-19 Sweet extract. All 3 events resolved without treatment. The study investigator considered each event to be unrelated to treatment. No clinically significant changes occurred in any of the other parameters with the exception of body temperature which tended to increase after ingestion.

This randomised, placebo controlled, double blind single dose study showed that 15 or 30 mg CH-19 Sweet extract/subject (equivalent of 3.96 mg/subject and 7.92 mg/subject of DHC, respectively or 0.066 mg DHC/kg bw and 0.132 mg DHC/kg bw assuming a mean bodyweight of 60Kg) was safe and well tolerated in healthy male volunteers.

2.2 12-week capsule safety assessment study in healthy male and female volunteers (C.E.F Lee) Protocol No. CH19-001

The safety of up to 12 weeks repeated capsule dosing of CH-19 Sweet extract was evaluated in a total of 75 subjects, (36 male and 39 female) of between 28 and 60 years of age. The protocol used a randomized, placebo-controlled, double-blind, parallel group design. Subjects received at least 1 dose of the test agent, including 38 subjects who received placebo (Capsule Lot 18F112) and 37 who received CH-19 Sweet extract (Capsule Lot No. 18F113). Subjects randomized to active treatment received 6 mg CH-19 Sweet extract (equivalent of 1.5 mg of DHC) or the control

arm received 6 capsules per day containing only inactive ingredients. The study was conducted at Advance Biomedical Research, Inc., Hackensack, New Jersey, USA.

Safety assessments included vital signs, 12-Lead ECG, clinical laboratory testing and monitoring for adverse events. Data were analysed using analysis of covariance (ANCOVA) with significance at the 5% level (two sided).

The treatment groups were comparable with regard to mean and median age and ethnicity.

Dosing with CH-19 Sweet extract was safe and well-tolerated; all adverse events reported were mild to moderate in severity, no serious adverse events occurred and none of the subjects discontinued dosing due to adverse events.
The overall incidence of adverse events (AEs) was similar in the placebo (44.7%) and CH-19 Sweet extract (37.8%) groups. The most commonly reported AEs were headache (15.8% in the placebo group and 5.4% in the CH-19 Sweet extract group) and nasopharyngitis (5.3% and 5.4%, respectively). All other AEs reported during the study occurred in only 1 or 2 subjects overall.

Gastrointestinal disorders appeared to occur more frequently in the CH-19 Sweet extract group subjects compared to placebo subjects. A total of 4 (10.8%) of 37 subjects in the CH-19 Sweet extract group experienced adverse events associated with the gastrointestinal system compared to none of the subjects in the placebo group, including dyspepsia (2 subjects), irregular bowel movement (1 subject) and diarrhoea (1 subject). All gastrointestinal complaints were mild to moderate in severity.

Adverse events assessed as possibly related to treatment were reported in 5 (13.2%) of 38 subjects in the placebo group and 7 (18.9%) of 37 subjects in the CH-19 Sweet extract group. Study agent related events in the placebo group were 3 reports of headache and 1 report each of neutropenia and myalgia. Study agent related events in the CH-19 Sweet extract group were 2 reports of dyspepsia and 1 report each of irregular bowel movement, diarrhea, thirst, migraine, and somnolence.

There were no clinically meaningful differences observed between the placebo and CH-19 Sweet extract groups with regard to changes from baseline for clinical laboratory parameters or vital signs.

It was concluded that CH-19 Sweet extract at a dose of 6 mg subject/day by capsule was safe and well tolerated over 84 days in males and females. This dosage was approximately equivalent to 1.5 mg DHC subject/day or 0.025 mg/kg bw/day (for a 60 kg human).

<u>Conclusion on Human Safety and Tolerance to CH-19 Sweet Extract</u>

Single dosage of CH-19 Sweet extract was found to be safe and well tolerated when administered to fasted male volunteers by capsule at 15 or 30 mg /subject. These doses were equivalent in terms of DHC content to 3.96 mg/subject and 7.92 mg/subject, respectively or 0.066 mg DHC/kg bw and 0.132 mg DHC/kg bw assuming a mean bodyweight of 60 Kg. Dosing of 6 mg CH-19 Sweet extract in capsules for 12 weeks to males and females, approximately equivalent to 0.25 mg DHC bw/day was again found to be safe and well tolerated with no clinical meaningful differences between placebo and CH-19 Sweet extract treated groups.

XIII.3 Potential Allergenicity Concerns for Humans

No allergenic reactions have been reported in workers involved in DHC or CH-19 Sweet extract production.

The food supplement Capsiate Natura™ marketed in Japan since 2006 and USA since 2007 contains CH-19 Sweet extract (7.5% capsinoids which contain DHC) see http://www.capsiatenatura.com/fda_letter.aspx.

Checking on the website AllergyNet, http://www.allallergy.net/fapaidfind.cfm?cdeoc=137

peppers have been found to cause allergy but there are no citations to adverse or indeed any effects resulting from DHC.

Based on the above information it seems that DHC which is synthesised and thus devoid of any source of plant material is unlikely to have the potential to cause IgE food related allergy.

Taking into account the synthetic process, a lipase, Novozyme® 435FG approved by the Danish Veterinary and Food Administration under MAFF (Appendix 1) is used for esterification. However the protein is immobilised on an inert carrier and studies have been made by Novozymes to show that the carrier is robust under normal usage with no release of the enzyme or other materials.

In consequence it it is considered unlikely that either the substance DHC or its production process are likely to result in food allergenicity by the novel food ingredient.

XIII.4 Safety Assessment

XIII.4.1 Determination of the no-observed-adverse-effect–level (NOAEL)

The NOAEL of 1000 mg/kg bw/day was derived from the 26-week oral gavage repeat dose toxicology in the rat conducted on commercial grade DHC as the study of longest duration. This was considered to be a reliable figure as the 13-week rat gavage repeat dose study and rat and rabbit teratology also resulted in the same NOAEL. All studies were conducted according to OECD guidance that requires frequent and thorough examination of the animals. The material tested was representative of commercial grade material (see Sub-section I.2 Table 2). The highest dose tested was the generally recognised international limit dose of 1000 mg/kg bw/day together with intermediate and low doses of 300 and 100 mg/kg bw/day.

XIII 4.2 Determination of the safety factor and safe level of intake

Traditional safety assessment utilises an appropriate ‘safety factor’ which is applied to the NOAEL derived from the most sensitive animal study, usually that giving the lowest NOAEL. As mentioned above the NOAEL was reproducibly found to be 1000 mg/kg bw/day. The only consistent findings were the presence of a mild increase in liver and kidney weight in the absence of any adverse histopathology with a sporadic and mild increase in transaminases in some animals. The findings were carefully evaluated and it was concluded that they probably reflect a physiological adaptation to bolus dosage of high levels of DHC (at the limit dose of 1000 mg/kg bw/day) and the resulting metabolic and excretory processes. Safety factors are normally derived in multiples of 10. A ten-fold factor is used for the extrapolation between animals and man (inter-species) and a further ten-fold factor for within species variation eg the very young to the very old (intra-species) resulting in an overall ‘safety factor’ of x100. In the event of additional uncertainty a further ten-fold uncertainty factor is applied to give an overall ‘safety factor’ of x1000. However, higher and lower values may be used according to the specific material in question. In the case of DHC there is a history of human consumption from chilli peppers without any evidence of adverse effects. Based on a thorough evaluation of the process and processing agents involved the material has an extremely low probability of any allergenic potential. There is no indication of toxicity or any toxicological or histophathological changes in the 13 or 26-week oral gavage studies in rats. Moreover DHC does not appear to be genotoxic.

In consequence it is reasonable to propose a safety factor of x100 from the NOAEL which results in a proposed safe level of intake of 10 mg/kg bw/day. This may be taken as a notional "acceptable daily intake" (ADI).

XIII 4.3 Margin of Safety for 'typical' and 'high-end' (97.5 percentile) consumers

On the basis that DHC may-be included in specified food items in 5 categories, baked goods, beverages, confectionary, cereals and deserts and other miscellaneous foods, where the potential to use DHC as a Novel Food ingredient exists, the Applicant is seeking approval for the use of 3 mg per portion. Actual use levels will vary between applications so that each portion of a given food product will deliver 3 mg of DHC. The actual concentration of DHC in any food item will therefore depend upon that manufacturer's product specification for single served products or on typical or recommended portion sizes for products presented in multi-served packs. The UK NDNS survey was analysed to determine the average quantity of food consumed at every eating occasion for each of the 5 potential food categories. The EU EFSA Concise Diets Database was also used to estimate mean intakes from each food category on a per capita basis.

Although it is anticipated that foods containing the Novel Food ingredient will normally be consumed by adults, intake estimates were also developed for all potential consumers including pre-school, school children and the elderly.

Table 18 below shows wide margins of safety for all groups including "worst-case" scenarios. All of the results are conservative and assume that consumers are eating all foods containing DHC all the time. In reality they will probably choose only certain foods containing DHC.

Table 18: Anticipated child and adult intakes of DHC for typical and high level consumers and Margins of Exposure

DHC Intake Level	Adult* exposure mg/kg bw/day	Child exposure mg/kg bw/day	Margin of Safety vs ADI of 10 mg/kg bw/day	
			Adult	Child
Mean daily intake	0.2	0.4	X50	X25
Average daily intake (95^{th} percentile)	0.4	1	X25	X10
High-end intake (EU Concise Database)	0.7	2	X14	X5
Natural DHC intake (maximum)	0.06	-	X166	-
Single serving using 3 mg DHC/portion	0.05	-	X200	-

*Assumes 60kg human

In conclusion, under a conservative set of assumptions, mean or average intakes of the DHC Novel Food ingredient are unlikely to exceed 0.2 to 0.4 mg/kg bw/day for an adult or 0.4 to 1.0 mg/kg bw/day for a child. Only a small percentage of the notional ADI is utilised resulting in a wide margin of safety.

CONCLUSION

DHC occurs naturally in a wide variety of edible non-pungent as well as pungent (hot) chilli peppers which have been consumed for centuries throughout the world. Chillis are eaten both raw and processed, alone and added to foods, for their flavour and sensory properties. DHC is a member of the capsinoid family, contained in certain *Capsicums,* which is remarkable by virtue of its lack of pungency compared with the capsaicinoids. DHC can be extracted from chillis and occurs in relatively high quantities (approximately 20%) in capsinoids which can be derived as an oily extract from CH-19 Sweet, a non-pungent cultivar of *Capsicum annuum*.

A chemical process developed by Ajinomoto results in the production of very pure DHC, which is manufactured to tight specifications under GMP and which is the subject of this novel food application.

The body of scientific evidence presented indicates that under the intended conditions of food use, DHC would not result in any adverse health effects. This conclusion is derived from an extensive safety programme conducted with both DHC and CH-19 Sweet extract (which contains DHC). Studies included toxicokinetics and toxicology as well as human clinical trials for hazard identification/characterisation and assessment of tolerance.

Exposure studies looked at the history of use of peppers and hence traditional background exposure to DHC (as a natural component of capsinoids) as well as the potential additional contribution from the use of DHC as a novel food ingredient. The repeat dose toxicology enabled the derivation of a no-observed–adverse-effect-level (NOAEL) from which a notional acceptable daily intake (ADI) of 10 mg/kg bw/day was calculated using a safety factor of X100. Using this figure a wide margin of safety was found to exist for typical adult and child consumers (Table 18 above). Even using a hypothetical worst case scenario for high-end (97.5 percentile) intake, good margins of safety exist for adults and children, based on the incorporation of DHC in a variety of foods at a standard inclusion rate of 3 mg/serving. This is equivalent to 0.05 mg/kg bw/day (3 mg/60 kg) for a 60 kg person. This represents a very conservative scenario as it is most unlikely that all of the identified foods in the 5 categories that are of interest to food manufacturers' will each be developed to contain DHC. Additionally the modelling assumes that consumers will be eating foods containing the DHC ingredient all the time whereas in reality the likelihood is that they will probably only target certain foods containing DHC. While there is inevitable variability in the data it was noteworthy that the UK (NDNS) and EU (Concise Diets Database) derived data showed reasonable concordance in terms of anticipated exposure. Moreover it was also interesting that UK consumers can be regarded as representative of typical higher level consumers in the broader EU.

Using modelling to develop data from limited publications in the field, it appears that natural DHC intake resulting from chilli pepper consumption could be up to 0.06 mg/kg bw/day. This potential background intake of natural DHC is closely comparable with the Ajinomoto DHC intake that would result for an adult from a single serving of food containing 3 mg DHC/portion, ie 0.06 mg/kg bw/day v 0.05 mg/kg bw/day. To place this in context, the estimated typical daily intake of an adult to DHC from 4 daily servings of food each containing 3 mg/portion would be 12 mg/day, equivalent to 0.20 mg/kg bw/day for a 60 kg human, which is only some 4X the high-end background natural exposure to DHC from chilli peppers.

Studies in human volunteers have investigated bolus doses of up to 12 mg DHC/volunteer for 8 days or 9 mg DHC/volunteer for 4 weeks in the absence of any obvious group related or clinically significant adverse effects. The individual adverse events that were recorded did not occur in more than 2 individuals, were not dose related or also were seen in the placebo control group. CH-19 Sweet extract was subjected to single dose and 12-week repeat dose administration. The maximum dose in terms of DHC in the 2 studies was approximately 8 mg/volunteer and 1.5 mg/volunteer, respectively. CH-19 Sweet extract is sold as a food supplement as Capsiate Natura™ in 3 mg soft gel capsules in the United States of America (USA) and in Japan.

In conclusion, the long history of use of chilli peppers coupled with the extensive toxicology, toxicokinetics and human volunteer studies supports the safety of DHC as a novel food ingredient for the European Community. The substance was granted GRAS status for use at 1 and 3 mg standard serving in the USA, in 2009 and 2010 respectively. Based on the exposure assessment resulting from

anticipated use, and taking into account the safety data, it is concluded that DHC does not represent a significant risk for human health at the proposed intake level, resulting from its intended use in food.

GLOSSARY

ADI – Acceptable Daily Intake

AE – Adverse Event

E-Capsiate - E-Capsiate is, with Z-capsiate, one of two optical isomers of capsiate, one of the 3 members of the capsinoid family. Only E-capsiate appears to occur in nature.

Capsiate Natura^{tm} – CH-19 Sweet extract (trademark name of marketed sweet pepper extract)

Capsinoids – a family of related substances principally including dihydrocapsiate, nordihydrocapsiate and capsiate, qv E-capsiate

CAS – Chemical Abstracts Service No.

CFSAN – Centre for Food and Drug Safety and Applied Nutrition

CH-19 SWEET EXTRACT – An oily extract from the Sweet Pepper CH-19 containing dihydrocapsiate, nordihydrocapsiate and capsiate

CoA – Certificate of Analysis

DHC – Dihydrocapsiate

EAFUS – Everything Added to Food in the United States

EFSA – European Food Standard Authority (Europe)

FAOSTAT – Food and Agriculture Organisation Statistical Service (UN)

FBS – Food Balance Sheet

FCC – Food Chemicals Codex

FDA – Food and Drug Administration (USA)

FSA – Food Standards Agency (UK)

GC – Gas Chromatography

GI – Gastrointestinal

GRAS – Generally Recognised As Safe (USFDA status term for foods having a demonstrated history of safe use)

HPLC – High Pressure Liquid Chromatography

IR – Infra-red

JP – Japanese Pharmacopoeia

JSFA – Japan's Specifications and Standards for Food Additives

MCT – Medium Chain Triglyceride

MNA – 8-Methyl Nonanoic Acid

MOE – Margin Of Exposure

MOS – Margin Of Safety

MSDS – Material Safety Data Sheet

NDIN – New Dietary Ingredient Notification (USFDA term)

NDNS – National Diet and Nutritional Survey (UK)

NOAEL – No-observed-adverse-effect-level

NOEL – No-observed-effect-level

PAFA – Priority-based Assessment of Food Additives

PWG – Pathology Working Group

SCF – Scientific Committee on Food

V- OH – Vanillyl Alcohol

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Appendix 0

USFDA Agency response of 'no objection' to GRAS Status for DHC, and USFDA and EU Countries 'no objection' for CH-19 Sweet extract

No Objection Letters	File Identifier
DHC – 1 mg DHC Portion/Standard Serving USA FDA "No objection" GRAS Notice No. GRN 000249 (2009)	DHC 1mg_ FDA No Objection Letter 090309.pdf
DHC – 3 mg DHC Portion/Standard Serving USA FDA "No objection" GRAS Notice No. GRN 000312 (2010)	DHC 3mg_FDA No Objection Letter 100606.pdf
CH-19 Sweet Extract - USA FDA "No objection" to New Dietary Ingredient status (2007)	CH-19 Sweet extract FDA_no objection letter.pdf
Czech Republic "No objection" Notification Letter (2008)	CH-19 Notification Letter-Czech.pdf
French Republic "No objection" Notification Letter (2009)	CH-19 Notification Letter-DGCCRF (France).pdf

Appendix 1

Material Safety Data Sheets (MSDS), Certificates of Analysis (CoA) and Analytical Methods

Material/Method	File Identifier
Commercial Grade DHC Material Safety Data Sheet (MSDS)	DHC MSDS.pdf
Commercial Grade DHC Certificates of Analysis (CoA)	DHC (CG) CoA Lot 060807.pdf DHC (CG) CoA Lot 070813.pdf
Laboratory Scale DHC Certificates of Analysis (CoA)	DHC (LS) CoA WKU05137ZBa.pdf
DHC Analytical Methods	DHC Analytical Methods.pdf
V-OH Material Safety Data Sheet (MSDS)	V-OH_4-Hydroxy-3-methoxybenzyl alcohol 98% MSDS.pdf
V-OH Certificate of Analysis	V-OH_COA.pdf
MNA Material Safety Data Sheet (MSDS)	8-Methylnonanoic acid MSDS.pdf
MNA Certificate of Analysis	8-MNA_COA.pdf
Novozyme® Free Sale Certificate	Novozym 435 FG_approval DK.pdf
Novozyme® Product Data Sheet	Novozym 435 FG product data sheet
Novozyme® Material Safety Data Sheet (MSDS)	Novozym435 MSDS.pdf
Medium chain triglyceride (MCT) Material Safety Data Sheet (MSDS)	ActorM2_MSDS_100506 (2).pdf
Medium chain triglyceride (MCT) Specification	ActorM2_Specification.pdf
CH-19 Sweet extract Certificates of Analysis	COA_eCap_2.pdf

Appendix 2

Exposure Data Tables

Sub-Appendix	File Identifier
Appendix 2.0	Capsicum intake NDNS
Appendix 2.1	NDNS Food codes for DHC Intake Estimates
Appendix 2.2	DHC intakes based on NDNS

Appendix 3

Study Reports on Commercial Grade DHC

Application ref # Section; Sub-section; Paragraph	Ajinomoto Study Reference	File Identifier
XIII 2.2. 1.1	AE-4518-G	DHC(CG)_1_PK.rat_AE-4518-G.pdf
XIII 2.3 1.1	07A003	DHC(CG)_2_Acute.rat_07A003.pdf
XIII 1.2.	C-B303	DHC(CG)_3_13-wk.rat_C-B303.pdf
XIII 1.3	C-B373	DHC(CG)_4_26-wk.rat_ cb373fr(E).pdf DHC(CG)_4_26-wk.rat_cb373am1(E).pdf
XIII 1.4	C-R060	DHC(CG)_5_Terato.rat_C-R060.pdf
XIII 1.5	C-R061	DHC(CG)_6_Terato.rab_C-R061.pdf
XIII 1.6	9994 (258-062)	DHC(CG)_7_Gene.mutation.rat_9994.pdf
XIII 1.7	A673 (258-066)	DHC(CG)_8_Micronucleus.mouse_A673.pdf
XIII 2.4 1.1	AMO-08-03	DHC(CG)_9_Human 8-day safety assessment_AMO-08-03.pdf
XIII 1.2	AMP-2008-06	DHC(CG)_10_Human 4-wk safety assessment_AMP-2008-06.pdf

Appendix 4

Study Reports on Laboratory Scale DHC

Ajinomoto Ref # Section; Sub-section; Para	Ajinomoto Study Ref	File Identifier
XIII 2.3 2.1	N-B205	DHC(LS)13-wk.rat_N-B205.pdf
XIII 2.2	9612 (258-046)	DHC(LS)Ames_9612.pdf
XIII 2.3	9613 (258-047)	DHC(LS)Chrom.Abberation_9613.pdf
XIII 2.4	9623 (258-048)	DHC(LS)Micronucleus.mouse_9623.pdf
XIII 2.5	9993 (258-061)	DHC(LS)Comet.Assay_9993.pdf

Appendix 5

Study Reports on CH-19 Sweet extract

Ajinomoto Ref # Section; Sub-section; Para	Ajinomoto Study Ref	File Identifier
XIII 2.2 2.1	XX05E-1103 XX05E-1002	Caps_1_Met-VOH.rat_XX05E-1103.pdf Caps_2_Met&PK.Rat_XX05E-1002.pdf
XIII 2.2	XX05E-104	Caps_3_P450study_XX05E-0104.pdf
XIII 2.3	PBC043-011	Caps_4_PK.SD-human_PBC043-011.pdf
XIII 2.3 3.1	N-B143	Caps_5_acute.rat_N-B143.pdf
XIII 3.2	N-B180	Caps_6_13-wk.rat_N-B180.pdf
XIII 3.3	N-B145	Caps_7_26-wk.rat_N-B145.pdf
XIII 3.4	N-R013	Caps_8_terat.rat_N-R013.pdf
XIII 3.5	N-R010	Caps_9_terato.rab_N-R010.pdf
XIII 3.6	N-R008	Caps_10_2-gen.rat_N-R008.pdf
XIII 3.7	9224 (258-041)	Caps_11_Ames_9224.pdf
XIII 3.8	9225 (258-042)	Caps_12_Chrom.Aberration_9225.pdf
XIII 3.9	9226 (258-043)	Caps_13_Micronucleus.mouse_9226.pdf
XIII 2.4 2.1	F0603	Caps_14_SD-Human tol_F0603.pdf
XIII 2.2	CH19-001*	Caps_15_Human 12-wk safety assessment.pdf

*All **Data Listings** referred to in Section 16 Appendix as pdf are attached as individual pdfs commencing L16_ immediately following study CH-19-001

*All **Tables** referred to in Section 14 as pdf are presented in this Appendix as individual pdfs commencing T14_ immediately following study CH-19-001

Appendix 6

International Journal of Toxicology Publications 2008 & 2010

International Journal of Toxicology Volume 27, Supplement 3, 2008
International Journal of Toxicology Volume 29, Supplement 1, 2010

Descriptor/Publication Title	Study No	File Identifier
Single-Dose Toxicity Study and Genotoxicity Studies of CH-19 Sweet Extract.	I	IJT#I.pdf
A 26-week Daily Gavage Dosing Toxicity Study of CH-19 Sweet Extract in Rats	II	IJT#II.pdf
A Two-Generation Reproduction Study of CH-19 Sweet Extract in Rats	III	IJT#III.pdf
Teratology Studies of CH-19 Sweet Extract in Rats and Rabbits	IV	IJT#IV.pdf
Genotoxicity Studies of Dihydrocapsiate	V	IJT#V.pdf
Single-Dose Toxicity Study and Micronucleus Test of Commercial-Grade Dihydrocapsiate	VI	IJT#VI.pdf
A 13-week Toxicity Study of Dihydrocapsiate in Rats	VII	IJT#VII.pdf
A 13-week Toxicity Study of Commercial-Grade Dihydrocapsiate in Rats	VIII	IJT#VIII.pdf
Teratology Studies of Dihydrocapsiate in Rats and Rabbits	IX	IJT#IX.pdf
Safety Assessment and Pharmacokinetics of Capsinoids in Healthy Male Volunteers after a Single Oral Ingestion of CH-19 Sweet Extract.	X	IJT#X.pdf
Pharmacokinetic and Tissue Distribution Study of C-Dihydrocapsiate and Metabolites in Rats	XI	IJT#XI.pdf
Pharmocokinets Study of Capsinoid-Containing CH-19 Sweet Extract in Rats	XII	IJT#XII.pdf
Inhibitory Effects of Capsaicin and Capsinoids on Cytochrome P450 3AE in Human Liver Microsomes	XIII	IJT#XIII.pdf
A 26-week Gavage Toxicity Study of Dihydrocapsiate in rats	XIV	IJT#XIV.pdf