APPLICATION FOR THE APPROVAL OF KIWIBERRY CONCENTRATE PRODUCED FROM THE HARDY KIWI (*ACTINIDIA ARGUTA*) FOR USE IN FOOD

Efficas, Inc.
7007 Winchester Circle, Suite 120
Boulder, CO, USA
80301

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Table of Contents

List of Appendices

List of Tables and Figures

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EXECUTIVE SUMMARY

KiwiBerry Concentrate is produced by concentrating the hot water soluble components of dried hardy kiwi fruit, *Actinidia arguta*, also known as baby kiwi fruit. It is intended as a "novelty fruit" ingredient in a variety of food products. Hardy kiwi fruit is similar in taste to the common green kiwi fruit, *Actinidia deliciosa*, but is smaller with a fuzzless, smooth skin. *A. arguta* is indigenous to northern China, Japan, Korea, and Siberia, and also is cultivated in these countries and has a documented history of human consumption. The fruit has been grown on a recreational and small scale in Europe. Populations commonly consuming this fruit in Siberia are primarily of European heritage.

A. arguta is a naturally occurring plant and has not been genetically modified. KiwiBerry Concentrate falls under category (e) of Article 1(2) of Regulation (EC) No 258/97 (European Parliament and the Council of the European Union, 1997) (foods and food ingredients consisting of or isolated from plants and food ingredients isolated from animals, except for foods and food ingredients obtained by traditional propagating or breeding practices) and is further classified under the European Commission's Scientific Committee on Food (SCF) Recommendations (Commission Recommendation 97/618/EC) (Commission of the European Communities, 1997), as a Class 2.2 [complex (non-GM derived)] Novel Food (NF), where the source of the NF has no history of food use in the Community.

The manufacturing process of KiwiBerry Concentrate consists of concentrating the hot water soluble components of dried hardy kiwi fruit (*A. arguta*) followed by filtration and evaporative concentration. The end product may be in the form of a liquid concentrate (KiwiBerry Liquid Concentrate) or be dried to produce a powdered concentrate (KiwiBerry Powder Concentrate). The production process has been shown to be tightly controlled and highly reproducible. A detailed compositional analysis has been presented.

Under the conditions of intended use, the percentage of KiwiBerry Concentrate users was high among all age groups evaluated. Greater than 91.4% of the population groups were estimated to consist of users of those food products in which KiwiBerry Concentrate is proposed for use. The population group with the greatest percentage of users was that of young people at 99.6%. Young people were determined to have the greatest mean all-user intake of KiwiBerry Concentrate on an absolute basis of the individual population groups, with a value of 4.8 g/person/day, and male teenagers were determined to have the greatest 97.5th percentile all-user intake with a value of 12.0 g/person/day. Conversely, on a body weight basis, children were identified as having the highest intake of any population group, with mean and 97.5th percentile all-user KiwiBerry Concentrate intakes of 243 and 598 mg/kg body weight/day, respectively. An average kiwifruit (*Actinidia deliciosa*) weighs

80 - 90 g, and at approximately 80% moisture, contains on the order of 7.2 g dry weight of solids. Thus, the 90th percentile consumption estimate for all-users is comparable to one serving of kiwifruit per day (range 0.7 - 1.18 servings per day), and the 97.5th percentile consumption estimate for all-users is comparable to 1.5 servings of kiwifruit per day (range 1.14 - 1.7 servings per day).

The safety of KiwiBerry Concentrate is supported by the proportional composition of the concentrate to the fresh hardy kiwi fruit itself as well as by the relationship and compositional similarity of hardy kiwi fruit to the common green kiwi fruit, which has enjoyed years of safe consumption by humans in the European Union (EU). Moreover, the safety of KiwiBerry Concentrate is substantiated by the composition of the ingredient, containing mainly carbohydrates, with minor amounts of protein and fat, and minimal levels of vitamins, minerals, and flavonoids, which all are expected to undergo normal metabolism. Additionally, various toxicology and clinical studies have been conducted on KiwiBerry Concentrate that support the safe use of the ingredient at level considerably higher than the anticipated intake, including a sub-choric day feeding study in the rat, conducted to GLP, which established a NOAEL for KiwiBerry Concentrate of 2,000 mg/kg bw/day, the highest dose tested. Allergenicty studies have also been conducted that indicate that the processing of KiwiBerry Concentrate would denature any potential allergenic proteins and the clear labelling of the product as being from Kiwi will provide further safeguard for consumers with a Kiwi fruit allergy.

The data and information summarised in this dossier demonstrate that KiwiBerry Concentrate, derived from hardy kiwi fruit, is safe for consumption as a food ingredient, based on history of use of green kiwi and corroborated by its relative equivalence to the green kiwi and by product-specific and other safety data, under the conditions of intended use in foods, as described herein.

1.0 ADMINISTRATIVE DETAILS

1.1 Name and Contact Details for Correspondence

Dr. Julianne Lindemann
Director of Regulatory Affairs
Efficas, Inc.
3260 Blume Dr., Suite 310
Richmond, CA, USA
94806
Telephone: 510-669-9496
Fax: 510-669-9951
Email: jlindemann@efficas.com

2.0 GENERAL INTRODUCTION

KiwiBerry Concentrate is produced by concentrating the hot water soluble components of the dried hardy kiwi fruit, *Actinidia arguta*, also known as baby kiwi fruit. Hardy kiwi fruit is similar in taste to the common green kiwi fruit, *Actinidia deliciosa*, but is smaller with a fuzzless, smooth skin (Strik and Cahn, 1996). Efficas, Inc. (Efficas) intends to market KiwiBerry Concentrate as a "novelty fruit" food ingredient in Europe in a variety of food products including beverages, cereal and cereal products, milk and milk products, sugars, preserves and confectionery, and savoury snacks. Approval is sought 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 (hereafter referred to as EC 258/97). Article 1(2.) of 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 and which fall under the following categories…(e) foods and food ingredients consisting of or isolated from plants and food ingredients isolated from animals, except for foods and food ingredients obtained by traditional propagating and breeding practices and which have a history of safe food use". KiwiBerry Concentrate is thus considered a novel food/food ingredient due to the isolation of the product from its source (European Parliament and the Council of the European Union, 1997).

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 EC 258/97 of the European Parliament and of the Council (Commission of the European Communities, 1997) (hereafter referred to as the Commission Recommendation of 1997), outlines the information required to support such applications. Specifically, in Section 4 of Commission Recommendation of 1997, the Scientific Committee on Food (SCF) outlines recommendations for the scientific classification of novel foods and divides them into six classes to facilitate the safety and nutritional evaluation of novel foods.

KiwiBerry Concentrate would be classified under Class 2 “Complex Novel Food from non-GM source”, and would be included in the sub-class 2.2 “the source of the Novel Food has no history of food use in the Community”, because the source of KiwiBerry Concentrate, the hardy kiwi fruit (*Actinidia arguta*), has not been consumed in significant amounts in the European Union; however, hardy kiwi has a history of consumption in Asia, and as *A. arguta* is very similar to the common green kiwi fruit, *A. deliciosa*, which also has a long history of human consumption, Section X “ Information from Previous Human Exposure to the Novel Food or its Source” has been included in this submission.

According to the SCF Recommendations described in Section 2.1, the following essential information is required:

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

X Information from Previous Human Exposure to the Novel Food or Its Source

XI Nutritional Information on the Novel Food

XII Microbiological Information on the Novel Food

XIII Toxicological Information on the Novel Food

In accordance with these recommendations, we present our submission in this required format.

I SPECIFICATION OF THE NOVEL FOOD

Based on Commission Recommendation 97/618/EC decision trees, the following questions must be addressed pertaining to the specifications of the novel food (Commission of the European Communities, 1997):

- "…is appropriate analytical information available on potentially toxic inherent constituents, external contaminants and nutrients?"
- "Is the information representative of the novel food when produced on a commercial scale?"
- "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?"

We will address each point in turn in this section.

A glossary is provided at the end of this document to provide explanation of abbreviated terms referred to in this dossier.

I.a Chemical Name

I.a.1 Common or Usual Name

Hardy kiwi concentrate

I.a.2 Trade Names

KiwiBerry Concentrate; KiwiBerry Powder Concentrate; KiwiBerry Liquid Concentrate;

I.a.3 Scientific Name and Common Synonyms

Actinidia arguta concentrate; arguta concentrate; kokuwa concentrate; tara vine concentrate; baby kiwi concentrate; cocktail kiwi concentrate; grape kiwi concentrate.

I.b Analytical Information

I.b.1 Physical and Chemical Characteristics of KiwiBerry Concentrate

KiwiBerry Concentrate is produced by concentrating the hot water soluble components of dried hardy kiwi fruit, with subsequent purification of the crude concentrate to produce the end product which may be in the form of a liquid concentrate (KiwiBerry Liquid Concentrate) or be dried to produce a powdered concentrate (KiwiBerry Powder Concentrate). The major components of hardy kiwi fruit, and hence the components of KiwiBerry Concentrate, are present in the common green kiwi fruit, *A. deliciosa*, although minor differences in relative amounts of the various constituents exist. Processing of *A. arguta* to produce KiwiBerry Concentrate does not disproportionately concentrate any particular component.

As mentioned, KiwiBerry Concentrate comprises mainly carbohydrates (at least 70%), with a minor amount of protein and fat (less than 10% of each), lesser amounts of ash (less than 8%), and also contains vitamin C and several minerals (calcium, magnesium, phosphorus, potassium, and sodium). Fibre levels are greater in fresh fruit than in KiwiBerry Concentrate, most likely due to the fact that most fibre components are not water soluble, and hence the filtration step will remove the insoluble fibre.

Fructose, glucose, inositol, and sucrose are the major sugars present in KiwiBerry Concentrate. Similar to the fresh fruit, flavonoids, such as quercetin, are present in KiwiBerry Concentrate, while anthocyanins also are present, but most are at levels much lower than identified in the fresh fruit because they are unstable in heat. The levels of organic acids present in KiwiBerry Concentrate are similar to those present in the fresh fruits. The composition of KiwiBerry Concentrate is discussed in detail in Sections I.b through I.d.

I.b.2 Additional Chemical Characterization

From a technical point of view KiwiBerry Liquid Concentrate is the ideal product form for compositional analysis, as it consists only of the KiwiBerry components and water. Thus, all of the analyses were conducted on 7 lots of KiwiBerry Liquid Concentrate. Three lots of

KiwiBerry Powder Concentrate were also subjected to a subset of the analytical tests to assure that the powders met the applicable specification. The content of phenolics and minerals for the powder concentrate can be calculated from the results of the liquid concentrate intermediate, based on dry weight blending ratios and known water content.

I.b.2.1 Organic Acids and Vitamin C

The organic acid and vitamin C content of 7 lots of KiwiBerry Liquid Concentrate were measured by Shuster Laboratories Inc. (Canton, MA) using United States Pharmacopoeia (USP) Method 28 and Association of Analytical Communities (AOAC) Official Methods of Analysis CG45, respectively. The amount of total organic acids ranged from approximately 100 to 144 mg/g, with citric acid being present at the highest level of all of the acids (approximately 70 to 83 mg/g), and lesser amounts of quinic acid (approximately 30 to 43 mg/g) and D-malic acid (approximately 11 to 22 mg/g). Vitamin C was present in the samples at a level of up to approximately 60 mg/kg. The results of these analyses are presented in Table I.b.2.1-1 and certificates of analysis are provided in Appendix I.

Table I.b.2.1-1 Results of Analysis of Organic Acid and Vitamin C Content of KiwiBerry Liquid Concentrate*

Parameter	Lot of KiwiBerry Liquid Concentrate (EFF-1001C)						
	FD001	SG05-0215A	SG05-0216A	SG05-0217A	SG05-0310-A	SG05-0311-A	SG05-0312-A
Citric acid (mg/g)	78.02	81.40	57.29	73.59	70.01	82.21	78.35
D-Malic acid (mg/g)	11.69	19.88	12.52	21.95	12.19	16.61	16.86
Quinic acid (mg/g)	34.74	42.21	31.15	39.59	32.23	42.42	41.36
Total Organic Acids (mg/g)	124.45	143.49	100.96	135.13	114.43	141.24	136.57
Vitamin C (mg/kg)	7.51	59.36[a]	5.98	2.30	8.26	0.99	3.28

* All measures are expressed on a dry weight basis
[a] Higher level is likely due to a difference in the ripeness of the fruit and/or normal agricultural variation.

Additionally, the organic acid and vitamin C content of 3 lots of KiwiBerry Powder Concentrate were measured by Shuster Laboratories Inc. (Canton, MA) using USP Method 28. The amount of total organic acids ranged from approximately 35 to 50 mg/g, with citric acid and quinic acid being present at similar levels, and lesser amounts of D-malic acid. Vitamin C was present in the samples at a level of up to 2.26 mg/g. The results of these analyses are presented in Table I.b.2.1-2 and certificates of analysis are provided in Appendix I.

Table I.b.2.1-2 Results of Analysis of Organic Acid and Vitamin C Content of KiwiBerry Powder Concentrate*			
Parameter	**Lot of KiwiBerry Powder Concentrate (EFF-1001P)**		
	FD001-P	**SG05-0215-P**	**SG05-0216-P**
Citric acid (mg/g)	15.52	19.23	18.19
D-Malic acid (mg/g)	6.58	10.34	9.61
Quinic acid (mg/g)	13.42	21.21	18.31
Acetic acid (mg/g)	0.01	0.01	0.01
Total Organic Acids (mg/g)	35.53	50.79	46.12
Vitamin C (mg/g)	1.01	2.26	1.58

* All measures are expressed on a dry weight basis

I.b.2.2 Phenolic Compounds

The flavonoid, anthocyanin, and catechin content of 7 lots of KiwiBerry Liquid Concentrate was analysed by ChromaDex, Inc. (Boulder, CO) using high-performance liquid chromatography (HPLC). Quercetin was determined to be the major flavonoid, and malvidin was determined to be the major anthocyanin. Catechins were not identified in any of the samples of KiwiBerry Liquid Concentrate. The results of these analyses are presented in Table I.b.2.2-1 and certificates of analysis are provided in Appendix I. Levels of the various phenolic compounds in the KiwiBerry Powder Concentrate can be calculated can be calculated from the results of the liquid concentrate intermediate on the basis of dry weight additions to dry inert carrier.

I.b.2.3 Mineral Components

KiwiBerry Liquid Concentrate contains low sodium levels, modest amounts of calcium, magnesium, and phosphorus, and high levels of potassium (Table I.b.2.3-1). These findings are consistent with the composition of *A. arguta* fruit from Oregon and published data from Japan (Okamoto and Goto, 2005). An average of the levels of these mineral components in 7 lots of KiwiBerry Liquid Concentrate, measured at The National Food Laboratory, Inc. (Dublin, CA) using AOAC Method 999.10, are presented in Table I.b.2.3-1 and certificates of analysis are provided in Appendix I. Levels of the various mineral components in the KiwiBerry Powder Concentrate can be calculated from the results of the liquid concentrate intermediate based on dry weight blending ratios and known water content.

Table I.b.2.3-1 Mineral Components of KiwiBerry Liquid Concentrate	
Mineral	**KiwiBerry Liquid Concentrate* (Mean ± SD)**
Calcium (mg/kg)	1,267.9 ± 365
Magnesium (mg/kg)	1,131.9 ± 241
Phosphorus (mg/kg)	1,993 ± 527
Potassium (mg/kg)	21,204 ± 1,062
Sodium (mg/kg)	338 ± 140

* Minerals are expressed as mean ± standard deviation of 7 independent lots of manufactured concentrate. Raw material for the KiwiBerry Concentrate was Oregon-sourced fruit from 2 years harvest.

I.b.3 Potentially Toxic Inherent Constituents

I.b.3.1 Kiwi Allergenicity

Kiwi fruit allergy presents a wide variety of symptoms ranging from localized oral allergy syndrome (OAS), most commonly characterized by itching and swelling of the lips, mouth, and throat, to more severe anaphylaxis (CFIA, 2000; Lucas *et al*., 2003). As with other food allergies, individuals with a known kiwi allergy can manage their condition by avoidance of kiwi.

I.b.3.2 KiwiBerry Liquid and Powder Concentrate

As described in further detail in Section XIII.c, samples of fresh hardy kiwi fruit (*i.e.,* non-reduced, unheated sample) and Efficas's KiwiBerry Liquid Concentrate (*i.e.,* reduced, heat-denatured sample) were evaluated using sera from 12 well-documented green kiwi-allergic individuals, and 2 of the individuals were reported to have immunoglobulin E (IgE) that binds to protein in fresh hardy kiwi fruit. With the exception of 1 donor, none of the donors with known green kiwi allergy were reported to have IgE bind to any protein in the KiwiBerry Liquid Concentrate. Allergens in hardy kiwi fruit are denatured by heat (Alemán *et al.,* 2004; Fiocchi *et al.,* 2004), and the authors reported that "the apparent lack of binding to proteins in processed hardy kiwi fruit concentrate, based on immunoblots, suggests that there is little likelihood that any of these kiwi fruit allergic individuals have IgE that binds specifically to protein present in the processed material"; however, the authors went on to say that the results were only based on *in vitro* data and therefore "caution must be exercised with respect to any broad recommendations regarding the allergenicity of heat-processed hardy kiwifruit concentrate for the entire population of kiwifruit-allergic consumers" (Chen *et al.,* 2006).

As mentioned, individuals with a known kiwi allergy can manage their condition by avoidance of kiwi. Since KiwiBerry Liquid and Powder Concentrates will be labelled as originating from hardy kiwi fruit, it is anticipated that individuals with kiwi allergy will avoid ingestion of these products. See Section XIII.c for further details.

I.b.4 External Contaminants

I.b.4.1 Pesticides and Fungicides

Hardy kiwi fruit is currently obtained from Oregon, U.S.A., and all fruit is produced under Good Agricultural Practices (GAP). Hardy kiwi is a perennial plant with a vining habit that is grown on support structures in a similar way to grapes. The harvested fruit, which is segregated from other crops, is clearly labelled, and is transported to a storage location and frozen within 72 hours of harvest. All of the pesticide materials applied to hardy kiwi plants and surrounding soil are registered for use on kiwi fruit according to the requirements of the U.S. Environmental Protection Agency (U.S. EPA), and residues of pesticides must be below established tolerance levels (if any) before the fruit is procured. Fungicides are not used during or after the bloom period due to a very low incidence of the fruit rotting fungus, botrytis. Ridomil Gold EC, whose active ingredient is metalaxyl, is applied post-harvest to control root rot in some fields, but never during the active growth period of the crop. An outline of the pesticide materials used during cultivation is provided in Appendix I.

A multi-residue analysis and a carbendazim analysis were carried out by an independent laboratory (Central Science Laboratory, UK). The results from the analyses indicated that no residues of the pesticides investigated (including metaxyl) were found in any of the samples, at or above the reporting limits quoted. The full report is provided in Appendix I.

I.b.4.2 Heavy Metals

The analysis for heavy metals is conducted at the liquid concentrate stage and calculations can be made for the dried powder made on the basis of moisture levels. Seven samples of KiwiBerry Concentrate were analysed by an independent laboratory (Central Science Laboratory, UK) for the presence of copper, arsenic, cadmium, mercury, and lead. Results for arsenic and mercury were below the limit of detection (0.05 and 0.005 mg/kg, respectively) for all 7 samples. Cadmium levels were also below the limit of detection (0.005 mg/kg) in all but one sample, which was above the limit of detection, but below the limit of quantification. Commission Regulation No. EC 466/2001 of 8 March 2001 sets maximum levels for certain contaminants in foodstuffs. The maximum level of cadmium permitted in fruits is 0.05 mg/kg. Under Regulation No. EC 466/2001, the maximum level of lead permitted in fruits is 0.1 mg/kg (Commission of the European Communities, 2001). The amount of lead in the 7 samples of KiwiBerry Concentrate analysed ranges from below the limit of quantification to 0.033 mg/kg (see Table I.b.4.2-1). The full report is provided in Appendix I.

Table I.b.4.2-1 Heavy Metal Analysis Results of KiwiBerry Concentrate								
Heavy Metal Tested (mg/kg)	**Maximum Level Permitted in Fruit* (mg/kg)**	**Batch Number**						
		SG05-0215A	**SG05-0216A**	**SG05-0217A**	**SG05-0310A**	**SG05-0311A**	**SG05-0312A**	**FD001 Powder**
Copper, Cu	ND	2.23	1.70	2.61	1.38	1.21	1.57	1.57
Arsenic, As	ND	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Cadmium, Cd	0.05	< 0.005	< 0.005	< 0.005	(0.005)	< 0.005	< 0.005	< 0.005
Mercury, Hg	ND	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Lead, Pb	0.2[#]	0.033	0.019	0.031	(0.010)	(0.009)	(0.016)	(0.007)

ND = not defined; Results given in brackets were above the limit of detection, but below the limit of quantification.
*Under Commission Regulation (EC) No 466/2001 of March 8 2001 (Commission of the European Communities, 2001)
[#]Maximum permitted in berries and small fruits as defined in Article 1 of Council Directive 90/642/EEC (Council of the European Communities, 1990)

I.b.4.3 Mycotoxin Analysis

Commission Regulation (EC) No. 472/2002 of 12 March 2002 amends Regulation (EC) No. 466/2001 setting maximum levels for certain contaminants in foodstuffs and sets a maximum level for ochratoxin A of 10 µg/kg in dried vine fruit. Six samples of KiwiBerry Liquid Concentrate and 1 sample of KiwiBerry Powder Concentrate were analysed by an independent laboratory (CSL) for the presence of ochratoxin A. Ochratoxin A levels were below 0.2 µg/kg in all 7 samples tested. The analyses were carried out to a reporting limit of 0.2 µg/kg. The full report is provided in Appendix I.

I.c Representative Commercial Scale Batch Data

Several lots of KiwiBerry Liquid Concentrate and KiwiBerry Powder Concentrate were analysed to verify that the manufacturing process produced a consistent product within the product specifications. A summary of the chemical and microbiological product analysis for 7 lots (FD001, SG05-0215A, SG05-0216A, SG05-0217A, SG05-0310-A, SG05-0311-A, and SG05-0312-A) of KiwiBerry Liquid Concentrate is presented in Table I.c-1 and demonstrates compliance with final product specifications. Since the KiwiBerry Powder Concentrate is obtained by drying the KiwiBerry Liquid Concentrate using conventional methods, and appropriate storage conditions will be employed, analysis was only performed on 3 lots of KiwiBerry Powder Concentrate (FD001-P, SG05-0215-P, and SG05-216-P). A summary of the chemical and microbiological product analysis for KiwiBerry Powder Concentrate is presented in Table I.c-2 and demonstrates compliance with final product specifications. See Appendix I for a summary of the analyses, certificates of analysis, and analytical methods.

Table I.c-1 Summary of the Chemical and Microbiological Product Analysis for 7 Lots of KiwiBerry Liquid Concentrate

Specification Parameter	Specification	Lot of KiwiBerry Liquid Concentrate (EFF-1001C)						
		FD001	SG05-0215A	SG05-0216A	SG05-0217A	SG05-0310-A	SG05-0311-A	SG05-0312-A
Moisture (%)	<50	42.71	30.59	28.09	30.29	26.15	39.18	35.93
Carbohydrate (%)[a]	>70	87.31	90.52	84.66	81.49	86.32	85.98	83.97
Protein (%)[a]	<10	5.80	4.74	6.06	5.87	6.23	6.63	6.07
Ash (%)[a]	<8	4.19	4.74	3.95	4.72	4.51	5.15	5.21
Fat (%)[a]	<10	2.71	<0.72	5.33	7.92	2.94	2.25	4.74
Total organic acids (mg/g)[a]	>50	124.45	143.49	100.96	135.13	114.43	141.24	136.57
Total heavy metals (mg/kg)[b]	<10	<10	<10	<10	<10	<10	<10	<10
Lead (mg/kg)[a]	<1	ND	0.033	0.019	0.031	(0.010)	(0.009)	(0.016)
Microbiological Parameters								
Total aerobic count (CFU/g)	≤10,000	≤10	≤10	≤10	≤10	≤10	≤10	≤10
Coliforms (MPN/g)	<3	<3	<3	<3	<3	<3	<3	<3
Escherichia coli (MPN/g)	<3	<3	<3	<3	<3	<3	<3	<3
Salmonella spp.	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Moulds (CFU/g)	≤500	<10	<10	<10	<10	<10	<10	<10

ND = not determined; Number in brackets represent results that were above the limit of detection (0.005 mg/kg) but below the limit of quantification.
[a] Measured on a dry weight basis
[b] Method detection limit (MDL) was 10 mg/kg

Table I.c-2 Summary of the Chemical and Microbiological Product Analysis for 3 Lots of KiwiBerry Powder Concentrate				
Specification Parameter	**Specification**	**Lot of KiwiBerry Powder Concentrate**		
		FD001-P	**SG05-0215-P**	**SG05-0216-P**
Moisture (%)	<6	2.12	0.94	1.03
Carbohydrate (%)[a]	>75	91.45	91.53	91.41
Protein (%)[a]	<5	2.84	3.21	3.30
Ash (%)[a]	<4	1.81	2.62	2.47
Fat (%)[a]	<5	1.78	1.70	1.79
Total organic acids (mg/g)[a]	>25	>25	>25	>25
Total heavy metals (mg/kg)[b]	<10	<10	<10	<10
Lead (mg/kg)[a]	<1	<1	<1	<1
Microbiological Parameters				
Total aerobic count (CFU/g)	≤10,000	≤10	≤10	50
Coliforms (MPN/g)	<3	<3	<3	<3
Escherichia coli (MPN/g)	<3	<3	<3	<3
Salmonella spp.	Negative	Negative	Negative	Negative
Moulds (CFU/g)	≤500	<10	<10	<10

[a] Measured on a dry weight basis
[b] Method detection limit (MDL) was 10 mg/kg

The product specifications for the KiwiBerry Liquid Concentrate indicate a carbohydrate level of >70% on a dry weight basis, which would correspond to approximately 35% carbohydrate on an as is basis. A quantitative analysis of the carbohydrates present in KiwiBerry Liquid Concentrate was conducted to determine the carbohydrate profile of the product. The sugars present in the product were measured by Medallion Laboratories using the approved official method of the AOAC, Method 977.20. The amount of starch and fibre present in KiwiBerry Liquid Concentrate were measured by Shuster Laboratories, Inc. (Canton, MA) using validated and approved official methods of the AOAC (Method 920.44 and Official Methods of Analysis, 17th Edition, 2000, CH45, p 78, respectively). See Appendix I for a summary of the analyses and analytical methods.

The results of the analysis indicated that sugars comprise the majority of the carbohydrate content of the ingredient, with lesser amounts of fibre and starch. The main sugars present in KiwiBerry Liquid Concentrate are fructose and glucose, with minor amounts of inositol and sucrose. A summary of the results of carbohydrate analysis of 7 consecutive lots of KiwiBerry Liquid Concentrate is presented in Table I.c-3.

Table I.c-3 Results of Analysis of Carbohydrate Composition of KiwiBerry Liquid Concentrate*							
Parameter	**Lot of KiwiBerry Liquid Concentrate (EFF-1001C)**						
	FD001	**SG05-0215A**	**SG05-0216A**	**SG05-0217A**	**SG05-0310-A**	**SG05-0311-A**	**SG05-0312-A**
Carbohydrate Component (g/100 g)							
Sugars	60.74	51.96	51.15	55.52	52.64	55.56	57.84
Starch	0.13	0.11	0.13	0.12	0.13	0.13	0.13
Fibre	3.49	6.92	3.48	5.16	5.55	4.60	4.99
Sugars (g/100 g)							
Fructose	30.02	25.07	24.06	26.83	25.32	26.80	28.87
Glucose	26.18	22.04	21.69	24.10	21.94	22.20	23.26
Inositol	4.54	4.75	5.15	4.59	4.87	5.59	5.46
Sucrose	0.00	0.10	0.25	0.00	0.51	0.97	0.25

* All measures are expressed on a dry weight basis

I.d Specification

The specifications for KiwiBerry Liquid Concentrate and KiwiBerry Powder Concentrate are presented in Tables I.d-1 and I.d-2, respectively. See Appendix I for details of the analytical methods and product specifications. As the KiwiBerry Concentrates are prepared from a natural source, several microbiological specifications similar to those performed for other food ingredients have been specified to ensure safety of its use in food. Various standard microbial tests appropriate for food ingredients are employed. The tests and limits also are presented in Tables I.d-1 and I.d-2.

Table I.d-1 Chemical and Microbiological Specifications for KiwiBerry Liquid Concentrate

Specification Parameter	Specification	Method of Analysis [a]
Moisture	<50%	AOAC Method 964.22
Carbohydrate	>70% (dry weight basis)	By difference[b]
Protein	<10% (dry weight basis)	AOAC Method 992.15
Ash	<8% (dry weight basis)	AOAC Method 940.262
Fat	<10% (dry weight basis)	Sample weight before and after supercritical fluid extraction with CO_2
Total organic acids	>50 mg/g (dry weight basis)	USP Method 28
Heavy metals	<10 mg/kg	USP Method 231
Lead	<1 mg/kg	AOAC Method 993.14 (ICP/MS)
Microbiological Parameters		
Total aerobic count	≤10,000 CFU/ g	AOAC Method 966.23
Coliforms	<3 MPN/g	U.S. FDA BAM, Ch. 4
Escherichia coli	<3 MPN/g	U.S. FDA BAM, Ch. 4
Salmonella spp.	Negative per 25 g	AOAC Method 996.08
Moulds	≤500 CFU/g	U.S. FDA BAM, Ch. 18

AOAC = Association of Analytical Communities; BAM = Bacterial Analytical Manual (Standard Methodologies) (U.S. FDA, 2001, 2002); CFU = colony forming units; CO_2 = carbon dioxide; ICP-MS = inductively-coupled plasma mass spectrometry; MPN = most probable number; USP = United States Pharmacopoeia

[a] Details of the analytical methodologies are presented in Appendix I

[b] By difference = 100% - (Fat% + Protein% + Ash%)

Table I.d-2 Chemical and Microbiological Specifications for KiwiBerry Powder Concentrate		
Specification Parameter	**Specification**	**Method of Analysis** [a]
Moisture	<6%	AOAC Method 964.22
Carbohydrate	>75%	By difference[b]
Protein	<5%	AOAC Method 992.15
Ash	<4%	AOAC Method 940.262
Fat	<5%	Sample weight before and after supercritical fluid extraction with CO_2
Total organic acids	>25 mg/g	USP Method 28
Heavy metals	<10 mg/kg	USP Method 231
Lead	<1 mg/kg	AOAC Method 993.14 (ICP/MS)
Microbiological Parameters		
Total aerobic count	≤10,000 CFU/g	AOAC Method 966.23
Coliforms	<3 MPN/g	U.S. FDA BAM, Ch. 4
Escherichia coli	<3 MPN/g	U.S. FDA BAM, Ch. 4
Salmonella spp.	Negative	AOAC Method 996.08
Moulds	≤500 CFU/g	U.S. FDA BAM, Ch. 18

AOAC = Association of Analytical Communities; BAM = Bacterial Analytical Manual (Standard Methodologies) (U.S. FDA, 2001, 2002); CFU = colony forming units; ICP-MS = Inductively coupled plasma mass spectrometry MPN = most probable number; NFL = National Food Laboratory, Inc.; USP = United States Pharmacopoeia
[a] Details of the analytical methodologies are presented in Appendix I
[b] By difference = 100% - (Fat% + Protein% + Ash%)

II EFFECT OF THE PRODUCTION PROCESS APPLIED TO THE NOVEL FOOD

Based on Commission Recommendation 97/618/EC decision trees, the following questions must be addressed pertaining to the production process of the novel food (Commission of the European Communities, 1997):

- "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 novel food compared to its traditional counterpart?"
- "Is information available to enable identification of the possible toxicological, nutritional and microbiological hazards arising from use of the process?"
- "Are the means identified for controlling the process to ensure that the novel food complies with its specification?"
- "Has the process the potential to alter the levels in the novel food of substances with an adverse effect on public health?"
- "After processing is the novel food likely to contain microorganisms of adverse public health significance?"

We will address each point in turn in this section.

II.a Raw Material Specifications

II.a.1 *Actinidia arguta* Fruit

Hardy kiwi fruit is currently obtained from Oregon, U.S.A., and all fruit is produced under GAP. The harvested fruit, which is segregated from other crops, is clearly labelled, and is transported to a storage location and frozen within 72 hours of harvest. All of the pesticide materials applied to hardy kiwi fruit are registered for use on kiwi fruit according to the requirements of the U.S. EPA, and residues of pesticides must be below established tolerance levels (if any) before the fruit is procured. Fungicides are not used during or after the bloom period due to a very low incidence of the fruit rotting fungus, botrytis. Ridomil is applied post-harvest to control root rot in some fields. An outline of the pesticide materials used is provided in Appendix I.

Prior to procurement, the fruit purchased for the production of KiwiBerry Concentrate must be in good and merchantable condition, free of all commercial defects, including but not limited to decomposition or decay of the berry induced by fungi, bacteria, or delay in delivery. No container of hardy kiwi fruit is accepted that contains >1.5% of material other than the fruit (*i.e.,* branches or insects) and/or 1.5% defects, such as crushed fruit.

Fresh or frozen hardy kiwi fruit is sliced (1/4" to 3/8" thickness) and then dried in a convection dryer (150 to 175°F) to a moisture content of 5 to 20%. Typical appearance of the dried fruit will be as dark brown chips, 2 to 3 cm in diameter, which are rubbery to crisp depending on their final moisture content.

II.a.2 Water

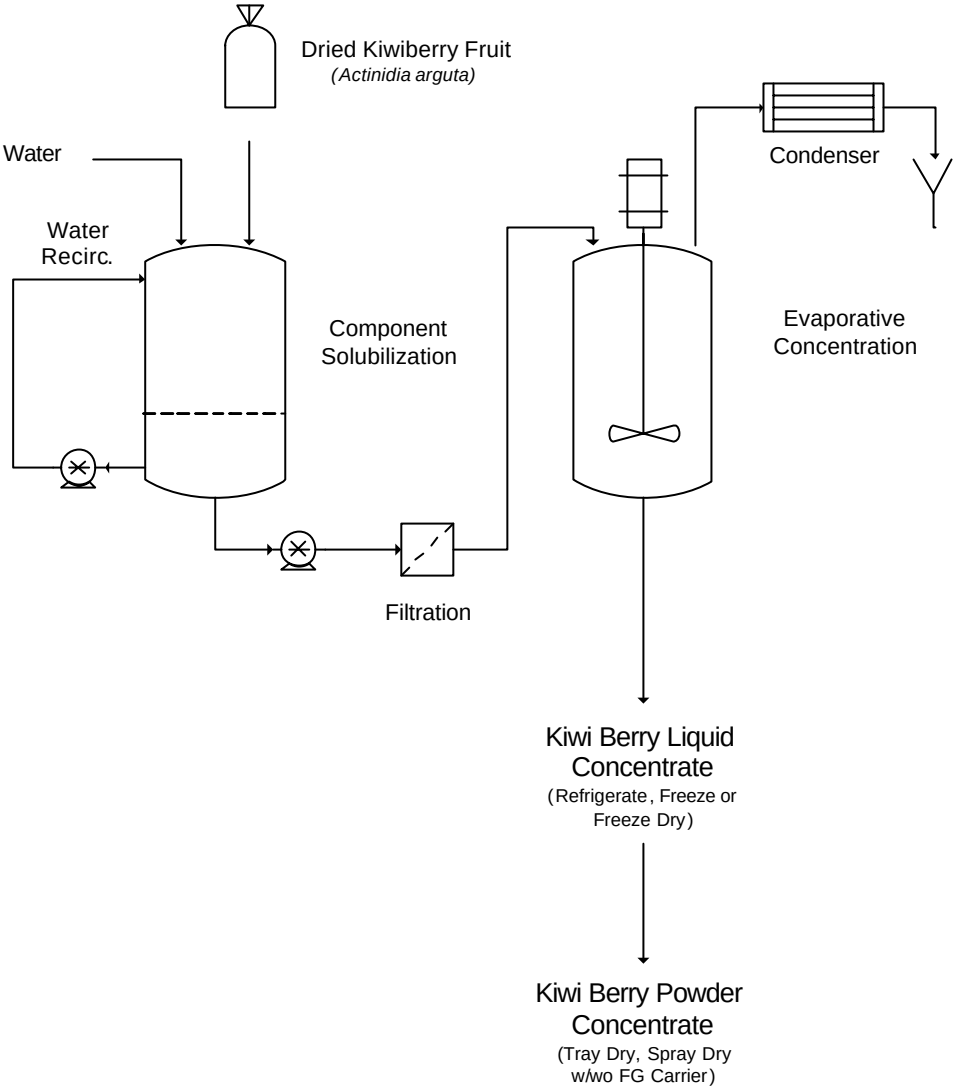
The water used in the manufacture of KiwiBerry Concentrate is potable water obtained from a regulated municipal water supply (see Appendix I).

II.b Manufacturing Process

As mentioned earlier, KiwiBerry Concentrate is produced from dried hardy kiwi fruit (*A. arguta*) by concentrating the hot water soluble components and removing insoluble components by filtration.

Briefly, the dried hardy kiwi fruit are harvested, sliced, dried, and cooked in hot water, and the insoluble components are removed by filtration. Water is the only solvent used in the process. The aqueous fruit concentrate (KiwiBerry Liquid Concentrate) may then be frozen, freeze-dried, or plated onto a dry inert carrier to produce a free flowing powder (KiwiBerry Powder Concentrate). Current good manufacturing processes (GMP) are employed during all processing operations. Additional details of the manufacturing process are provided in Sections II.b.1 through II.b.5 and in Appendix I. A schematic of the manufacturing process is presented in Figure II.b-1.

Figure II.b-1 KiwiBerry Concentrate Process Overview



II.b.1 Component Solubilization

Batch processing of the fruit biomass is performed in an unstirred jacketed stainless steel reactor with an internal filter screen (200 mesh) near the bottom of the tank to support the extraction load. Water is re-circulated through the biomass using an external recirculation loop. An external condenser is employed to prevent water loss during the solubilization operation. The contents of the vessel are gradually warmed from ambient temperature to a specific temperature *via* the introduction of steam into the jacket of the reactor. Following completion of the solubilization period, the spent biomass is removed for disposal and the aqueous material is advanced for further processing.

II.b.2 Filtration

The hot aqueous material is pumped through a filter for the removal of particulate matter. Following the filtration, the clarified material is directed to a second vessel for concentration.

II.b.3 Concentration

Concentration of the filtrate is carried out in a vacuum evaporator or an agitated stainless steel reactor equipped with an external condenser and a distillate receiver. Water is evaporated from the filtrate under vacuum. The final concentrate can be packaged as is and stored under refrigeration or freezer conditions.

II.b.4 Manufacture of the Powdered Product Form

Dry forms of the product can be produced by lyophilization of the wet extract, or the concentrate can be dried using conventional technologies such as spray drying or tray drying. It may or may not be necessary to use food-grade processing aids, such as microcrystalline cellulose or maltodextrin, to facilitate complete drying of the concentrate. Microcrystalline cellulose is permitted for use in foodstuffs as a food additive under Directive 95/2/EC following the *quantum satis* principle with no maximum level specified other than it shall not be used at a lever higher than is necessary to achieve the intended purpose (European Parliament and the Council of the European Union, 1995). Maltodextrin is a form of partially hydrolysed starch and is considered a food, thereby making it exempt from the food additives regulations. The dried product forms can be milled in order to achieve specific particle size requirements. The final KiwiBerry Powder Concentrate product is a tan to light brown free flowing powder.

II.b.5 Packaging and Release of the Final Product(s)

All finished product is packaged in appropriate food-grade packaging materials. In the case of the neat concentrate, glass or food-grade plastic jars and carboys are used. In the case of the powder forms of the product, fibre drums with double polyethylene liners are typically employed.

Representative samples of all product lots are tested by a qualified laboratory, using approved standard test procedures, to ensure that the material complies with the specification that has been set for this product. Final product release is based on a successful review of the product's manufacturing and testing records by an Efficas Quality Assurance representative, whose responsibility it will be to confirm that the product has been manufactured according to current GMP and that it meets the specification limits for the product.

II.c Stability of KiwiBerry Concentrate

The stability of KiwiBerry Powder Concentrate was analysed by Medallion Laboratories (Minneapolis, MN) under various storage conditions monthly for up to 3 months. The storage conditions that were simulated during these stability studies were as follows: 70°F,

38% relative humidity; 40°F, ambient relative humidity; and 100°F, 20% relative humidity. The KiwiBerry Powder Concentrate was packaged in double poly bags and samples were analysed monthly for sensory characteristics such as colour, clumping, and aroma, as well as analytical parameters such as pH, moisture, water activity (Aw), total sugars, and microbial content. The results of these stability studies indicated that KiwiBerry Powder Concentrate was very stable at room temperature and under accelerated conditions. A summary of the results of the studies is presented in Tables II.c-1 and II.c-2, and complete details are presented in Appendix II. Additionally, photographs of the KiwiBerry Powder Concentrate under the simulated storage conditions were taken at 0, 1, 2, and 3 months and further demonstrate the stability *via* sensory parameters (*i.e.,* a lack of discoloration throughout the study period) [conducted by Merlin Development, Inc. (Plymouth, MN)]. These pictures are presented in Figures II.c-1 to II.c-4.

Table II.c-1 Results of Analytical Assessment on KiwiBerry Powder Concentrate from the 3-Month Stability Study

Time	pH	Moisture (%)	Aw	Total Sugars (%)*	Fructose (%)	Glucose (%)	Sucrose (%)	Total Aerobic Microbes (CFU/g)
Baseline	3.72	1.24	0.166	16.3	8.84	7.46	0	<10
70°F, 38% Relative Humidity								
1 month	3.63	1.36	0.189	16.5	9.01	7.50	0	<10
2 months	3.73	1.36	0.178	19.6	8.88	7.48	0	<10
3 months	3.63	1.36	0.244	16	8.88	7.07	0	<10
40°F, Ambient Relative Humidity								
1 month	3.63	1.28	0.183	16.3	8.88	7.46	0	<10
2 months	3.72	1.20	0.196	19.6	8.82	7.45	0	<10
3 months	3.64	1.28	0.209	16	8.92	7.05	0	<10
100°F, 20% Relative Humidity								
1 month	3.63	1.20	0.157	16.3	8.9	7.42	0	<10
2 months	3.74	1.28	0.161	19.5	8.62	7.36	0	<10
3 months	3.65	1.36	0.221	16.5	8.76	7.78	0	<10

Aw = water activity; CFU = colony forming units
* Measured by high-performance liquid chromatography (HPLC). Total sugars (%) are lower than those measured for the KiwiBerry Liquid Concentrate due to the presence of the inert carrier(s) in the powder end product.

Table II.c-2 Sensory Assessment Results from a 3-Month Stability Study on KiwiBerry Powder Concentrate

Time	Hunter Colour*			Light/Dark	Red/ Brown	Clumping	Particle Size	Dried Fruit Aroma	Off Aroma
	L	a	b						
Baseline	56.8	10.7	25.9	40	25	5	20	30	2.5
70°F, 38% Relative Humidity									
1 month	55.5	10	24.9	40	25	5	20	30	2.5
2 months	56.4	9.82	24.6	40	26	5	20	30	2.5
3 months	54.5	10.1	25	41	27	5	20	25	2.5
40°F, Ambient Relative Humidity									
1 month	56.0	9.99	25	40	25	5	20	30	2.5
2 months	55.3	10.2	25.3	40	26	5	20	30	2.5
3 months	50.2	9.63	22.8	40	25	5	20	30	2.5
100°F, 20% Relative Humidity									
1 month	56.3	10.7	25.6	40	25	5	20	30	2.5
2 months	56.5	10.7	25.4	40	26	5	20	30	2.5
3 months	56.3	11.1	26	45	28	5	28	22	2.5

* L, a, and b represent axes on the Hunter Colour Scale. A full explanation of the Hunter Lab Colour Scale is provided in Appendix II.

Figure II.c-1 KiwiBerry Powder Concentrate at Time 0 of the Stability Study

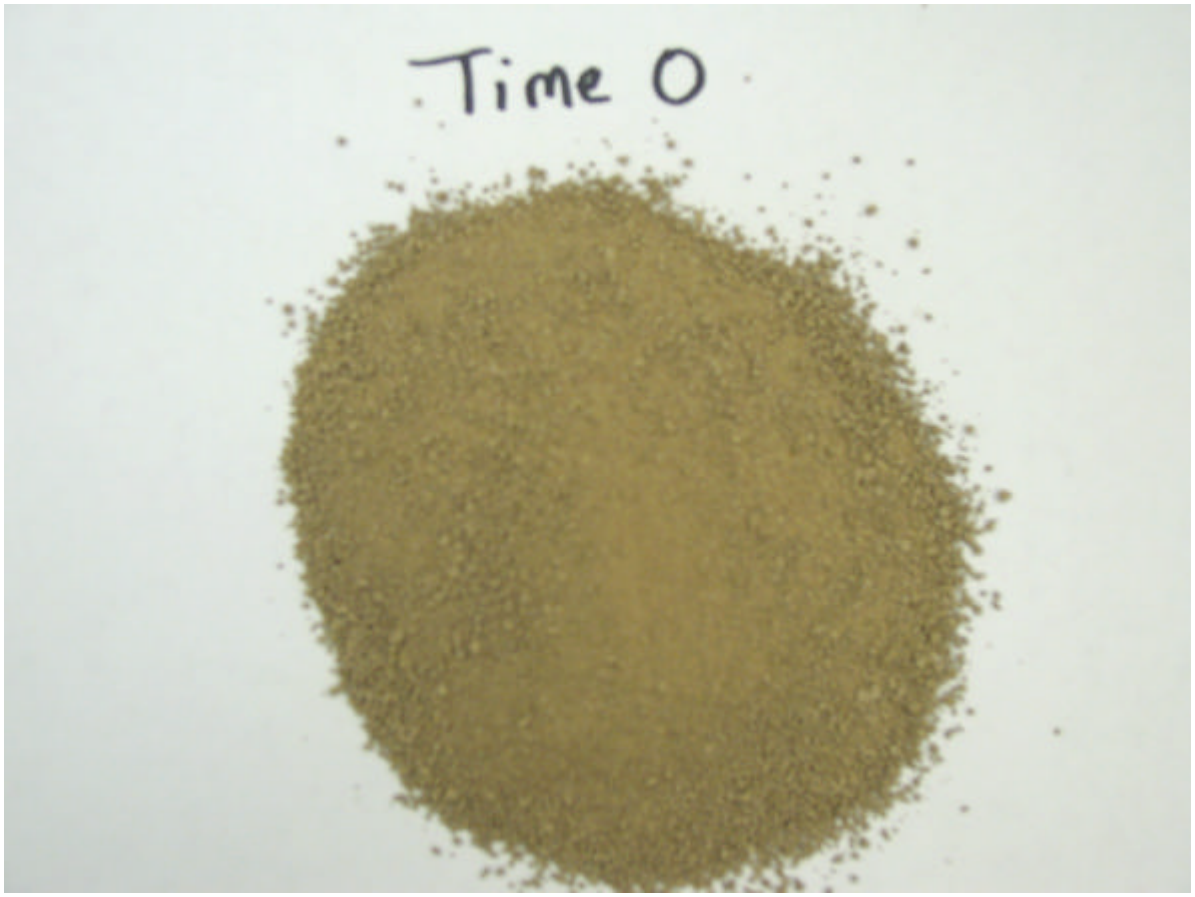


Figure II.c-2 KiwiBerry Powder Concentrate after 1 Month of the Stability Study

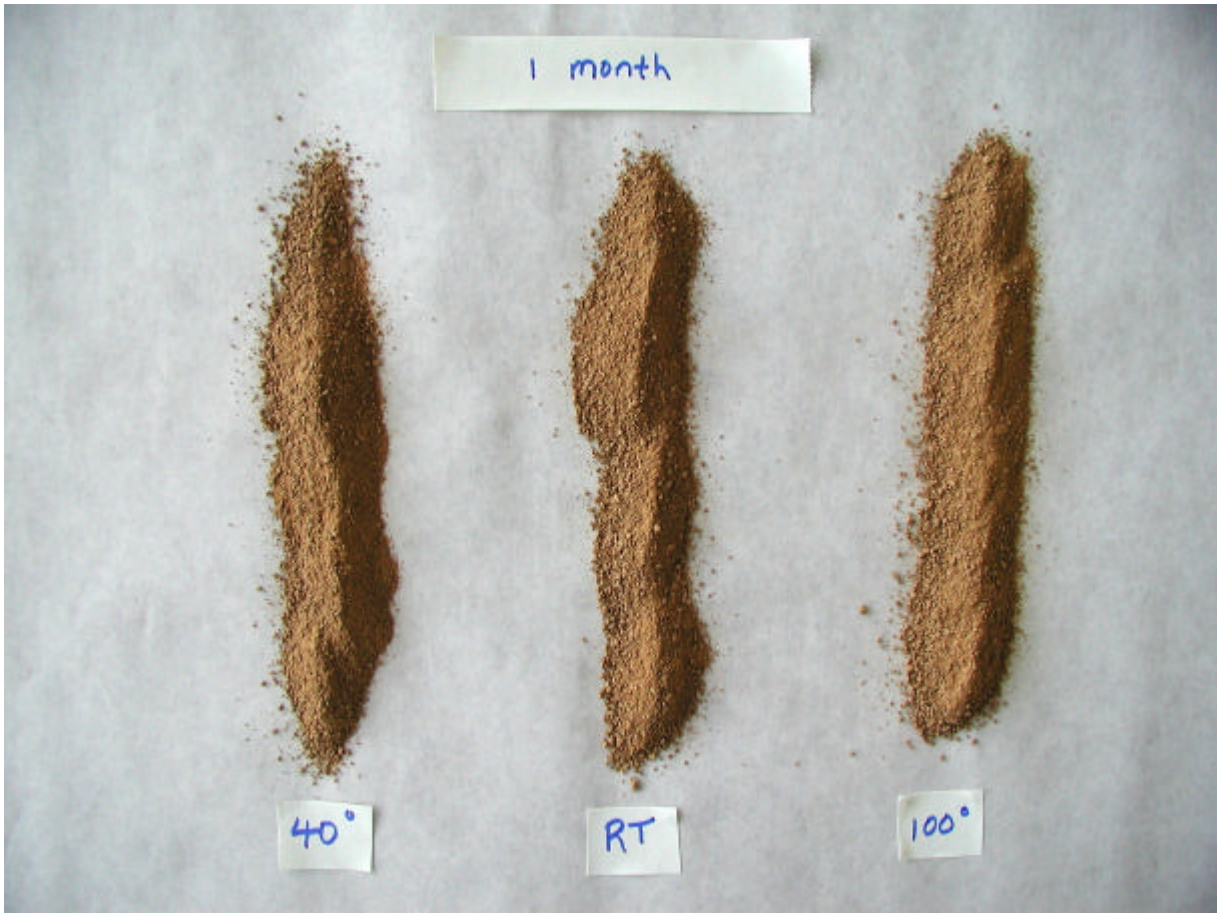


Figure II.c-3 **KiwiBerry Powder Concentrate after 2 Months of the Stability Study**

Figure II.c-4 **KiwiBerry Powder Concentrate after 3 Months of the Stability Study**

An additional study was conducted to investigate the stability of KiwiBerry Powder Concentrate when added to a granola bar. A granola bar product was developed by Merlin Development, Inc. (Plymouth, MN), the composition of which is outlined in Table II.c-3. An additional set of granola bars containing no KiwiBerry Powder Concentrate was used as a control. Finished granola bars were packaged in 3-ply pouches, consisting of a layer of polyester, aluminium foil, and cast polypropylene (Kapak model PFC17), materials that are commonly used to package items ranging from medical devices to food products. Each pouch contained 3 granola bars.

The stability of KiwiBerry Powder Concentrate in the granola bars was analysed by Medallion Laboratories (Minneapolis, MN) under various storage conditions for a period of 3 months. The storage conditions that were simulated during this stability study were as follows: 70°F, 38% relative humidity; 40°F, ambient relative humidity; and 100°F, 20% relative humidity. The control granola bars and bars containing KiwiBerry Powder Concentrate were analysed after 3 months for sensory characteristics such as colour,

clumping, and aroma, and analytical parameters such as pH, moisture, Aw, total sugars, and microbial content.

Table II.c-3 Composition of Control and KiwiBerry Powder Concentrate Granola Bars

Ingredient	% Composition of Original Recipe		% Ingredient in Final Product	
	Control Bar	KiwiBerry Bar	Control Bar	KiwiBerry Bar
Cereal Ingredients				
Natural granola cereal	25	25	15.00	15.00
Crisp rice	15	15	9.00	9.00
Whole Oats, Old Fashioned	28	28	16.80	16.80
Whole Oats, Quick Cooking	20	20	12.00	12.00
Raisins	12	12	7.20	7.20
TOTAL	100	100	--	--
Binder Syrup Ingredients				
Corn syrup, 32 – 48% high maltose	40.00	40.00	16.00	16.00
Sugar, fine	17.00	12.72	6.80	5.09
Corn syrup solids, 20 DE	14.40	10.11	5.76	4.04
KiwiBerry Concentrate powder	0.00	8.57	0.00	3.43
Raftilose P95 Inulin	5.00	5.00	2.00	2.00
High fructose corn syrup, 55%	8.00	8.00	3.20	3.20
Glycerine, 99% USP	7.00	7.00	2.80	2.80
Sorbitol	3.00	3.00	1.20	1.20
Salt	1.30	1.30	0.52	0.52
Vegetable shortening	4.00	4.00	1.60	1.60
Vanilla Extract	0.30	0.30	0.12	0.12
TOTAL	100.00	100.00	100.00	100.00

DE = dry extract; USP = United States Pharmacopoeia

Results of this stability study indicated that the addition of KiwiBerry Powder Concentrate to a granola bar product had no significant impact on the stability of the ingredient at room temperature or under accelerated conditions. The results of the study are presented in Tables II.c-4 and II.c-5, and complete study details are presented in Appendix II. Additionally, photographs of the granola bars under the simulated storage conditions at 0 and 3 months are presented in Figures II.c-5 and II.c-6 and show no or minimal change in colour throughout the study period, further supporting the fact that the KiwiBerry Powder Concentrate remains stable in the food matrix.

Table II.c-4 Results of Analytical Assessment on Control and KiwiBerry Powder Concentrate-Containing Granola Bars from the 3-Month Stability Study

Time	pH		Total Aerobic Microbes (CFU/g)		Total Sugars (%)*		Fructose (%)		Glucose (%)		Sucrose (%)		Maltose (%)**		Lactose (%)**	
	C	KBC	C	KBC	C	KBC	C	KBC	C	KBC	C	KBC	C	KBC	C	KBC
0 months	5.65	5.44	100	<100	31.5	28.9	5.8	4.38	7.01	5.69	9.51	8.21	9.22	10.6	0	0
70°F, 38% Relative Humidity																
3 months	5.75	5.28	<100	<100	30.4	27.7	4.33	5.33	5.51	5.90	9.70	7.56	9.99	8.52	0.91	0.42
40°F, Ambient Relative Humidity																
3 months	5.68	5.55	<100	<100	31.2	25.7	4.98	3.01	6.16	4.14	8.97	8.43	10.30	9.58	0.74	0.58
100°F, 20% Relative Humidity																
3 months	5.39	5.18	<100	<100	29.4	27.2	5.42	4.32	5.75	4.70	9.07	8.05	8.30	9.28	0.81	0.86

Aw = water activity; C = control bar; CFU = colony forming units; KCB = KiwiBerry Powder Concentrate-containing bar
* Measured by high-performance liquid chromatography (HPLC)
** Maltose and lactose are present as a result of the other ingredients used in the granola bar product formulation

Table II.c-5 Sensory Assessment on Control and KiwiBerry Powder Concentrate-Containing Granola Bars from a 3-Month Stability Study

Time	Colour		Flexibility		Firmness		Chewiness		Sweetness		Grain Flavour		Dried Fruit Flavour		Palate Burn*		Off Flavour	
	C	KBC	C	KBC	C	KBC	C	KBC	C	KBC	C	KBC	C	KBC	C	KBC	C	KBC
0 months	20.4	33	39	41.4	19.2	17	42	43.4	40.2	32.8	25	18.4	16	28.4	5.1	16.7	2.8	3.1
70°F, 38% Relative Humidity																		
3 months	21	36	35.8	30.8	20.8	22.2	31.4	32.8	32	27.2	34	29	14	15.4	9	13	4.8	8.4
40°F, Ambient Relative Humidity																		
3 months	18	32	40.4	34.6	17.4	16.4	31.2	28.8	33	29	34.6	28.6	13.4	16.6	9	13.6	4.4	7.8
100°F, 20% Relative Humidity																		
3 months	28.2	42	30.4	28.6	26.4	29.2	40.4	43.2	26.8	23.6	29.2	23	18	25.2	13	16	15.2	14.8

C = control; KBC = KiwiBerry Concentrate

* Palate burn is defined as degree of irritation on the roof of the mouth (Merlin Development Inc., 2005 [personal communication]).

Figure II.c-5 **Control and KiwiBerry Powder Concentrate-Containing Granola Bars at Time 0 of the Stability Study**

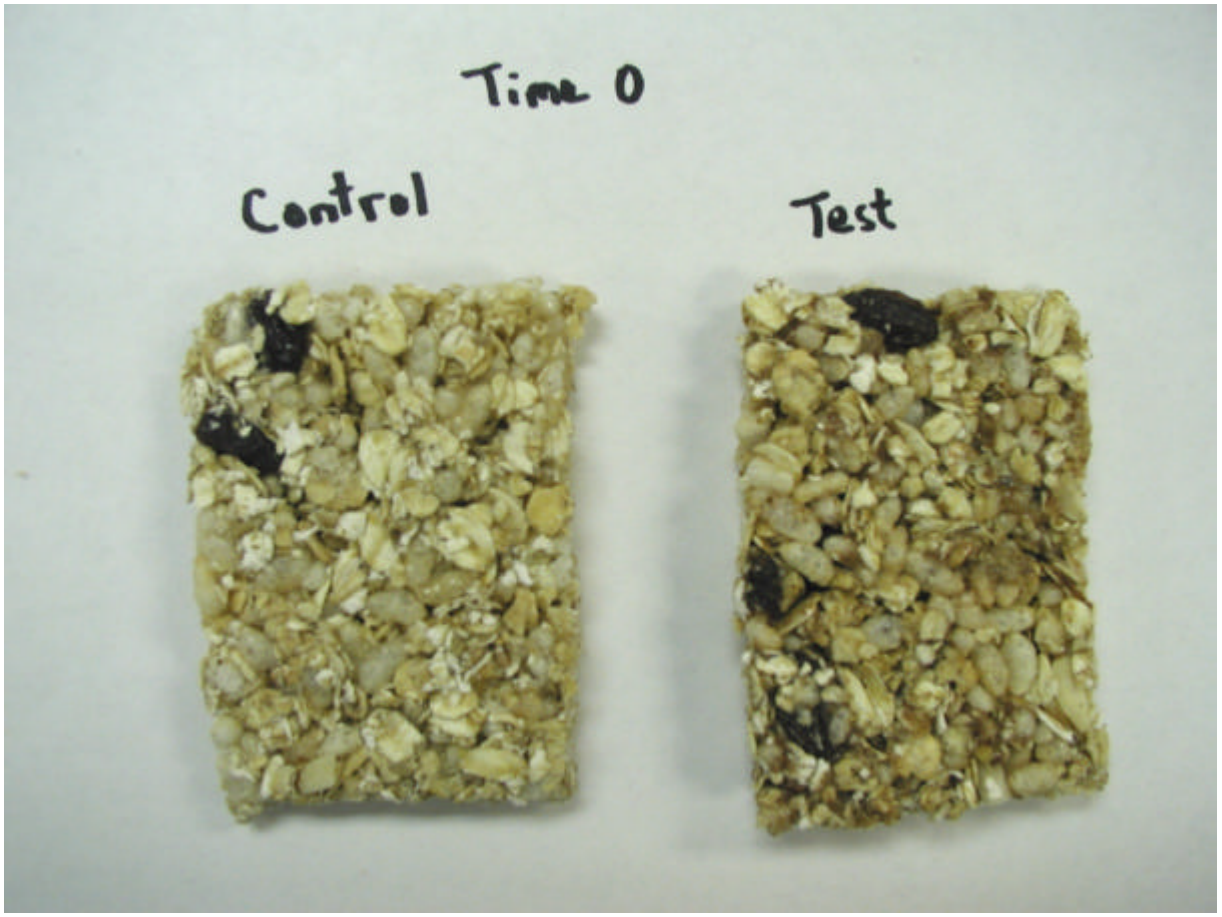
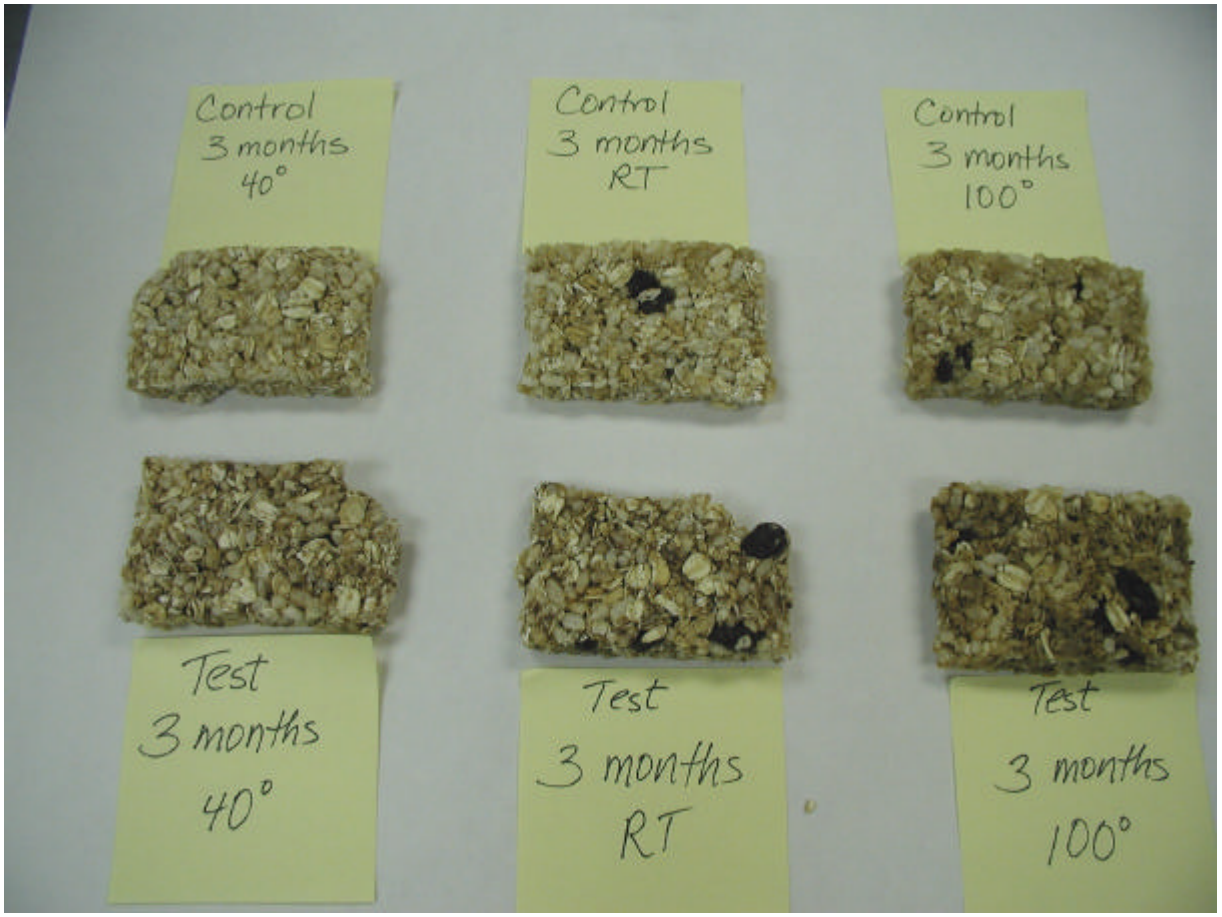


Figure II.c-6 Control and KiwiBerry Powder Concentrate-Containing Granola Bars after 3 Months of the Stability Study



II.d History of Production Process

Hot water extraction is a common production process that has been used for many years. Zhang *et al*. (1992) reported that fruit extracts of *A. arguta*, prepared by boiling dried fruits, have been used traditionally to improve digestion and general health in China.

Additionally, the major components of *A. arguta* and KiwiBerry Concentrate are present in the common kiwi fruit *A. deliciosa*, which has a long history of human consumption. Minor differences in relative amounts of the various constituents do not pose a hazard. Processing of *A. arguta* to produce KiwiBerry Concentrate does not disproportionately concentrate any particular component that would be a cause for concern. See Section XI for further details.

II.e Potential Hazards

II.e.1 Toxicological

See Section I.b.3 and XIII.c.

II.e.2 Nutritional

See Sections I.b.1 and I.b.2.

II.e.3 Microbiological

II.e.3.1 Microbiological Specifications for KiwiBerry Concentrate

The microbiological specifications for KiwiBerry Liquid Concentrate and KiwiBerry Powder Concentrate are presented in Tables I.c.3.1-1 and I.c.3.1-2, respectively. See Appendix I for details of the analytical methods and product specifications. As the KiwiBerry Concentrates are prepared from a natural source, several microbiological specifications similar to those performed for other food ingredients have been specified to ensure safety of its use in food. Various standard microbial tests appropriate for food ingredients are employed. The tests and limits also are presented in Tables II.c.3.1-1 and II.c.3.1-2.

Table II.e.3.1-1 Microbiological Specifications for KiwiBerry Liquid Concentrate

Specification Parameter	Specification	Method of Analysis [a]
Total aerobic count	≤10,000 CFU/ g	AOAC Method 966.23
Coliforms	<3 MPN/g	U.S. FDA BAM, Ch. 4
Escherichia coli	<3 MPN/g	U.S. FDA BAM, Ch. 4
Salmonella spp.	Negative per 25 g	AOAC Method 996.08
Moulds	≤ 500 CFU/g	U.S. FDA BAM, Ch. 18

AOAC = Association of Analytical Communities; BAM = Bacterial Analytical Manual (Standard Methodologies) (U.S. FDA, 2001, 2002); CFU = colony forming units; MPN = most probable number
[a] Details of the analytical methodologies are presented in Appendix I

Table II.e.3.1-2 Microbiological Specifications for KiwiBerry Powder Concentrate

Specification Parameter	Specification	Method of Analysis [a]
Total aerobic count	≤10,000 CFU/g	AOAC Method 966.23
Coliforms	<3 MPN/g	U.S. FDA BAM, Ch. 4
Escherichia coli	<3 MPN/g	U.S. FDA BAM, Ch. 4
Salmonella spp.	Negative	AOAC Method 996.08
Moulds	≤500 CFU/g	U.S. FDA BAM, Ch. 18

AOAC = Association of Analytical Communities; BAM = Bacterial Analytical Manual (Standard Methodologies) (U.S. FDA, 2001, 2002); CFU = colony forming units; MPN = most probable number
[a] Details of the analytical methodologies are presented in Appendix I

II.e.3.2 Microbiological Analyses for KiwiBerry Concentrate

Several lots of KiwiBerry Liquid Concentrate and KiwiBerry Powder Concentrate were analysed to verify that the manufacturing process produced a consistent product within the product specifications. A summary of the microbiological product analysis for 7 lots (FD001, SG05-0215A, SG05-0216A, SG05-0217A, SG05-0310-A, SG05-0311-A, and SG05-0312-A)

of KiwiBerry Liquid Concentrate is presented in Table II.c.3.2-1 and demonstrates compliance with final product specifications. Since the KiwiBerry Powder Concentrate is obtained by drying the KiwiBerry Liquid Concentrate using conventional methods, and appropriate storage conditions will be employed, analysis was only performed on 3 lots of KiwiBerry Powder Concentrate (FD001-P, SG05-0215-P, and SG05-216-P). A summary of the microbiological product analysis for KiwiBerry Powder Concentrate is presented in Table II.c.3.2-2 and demonstrates compliance with final product specifications. See Appendix I for a summary of the analyses, certificates of analysis, and analytical methods.

Table II.e.3.2-1 Summary of the Microbiological Product Analysis for 7 Lots of KiwiBerry Liquid Concentrate

Specification Parameter	Specification	Lot of KiwiBerry Liquid Concentrate (EFF-1001C)						
		FD001	SG05-0215A	SG05-0216A	SG05-0217A	SG05-0310-A	SG05-0311-A	SG05-0312-A
Total aerobic count (CFU/g)	≤10,000	≤10	≤10	≤10	≤10	≤10	≤10	≤10
Coliforms (MPN/g)	<3	<3	<3	<3	<3	<3	<3	<3
Escherichia coli (MPN/g)	<3	<3	<3	<3	<3	<3	<3	<3
Salmonella spp.	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Moulds (CFU/g)	≤500	<10	<10	<10	<10	<10	<10	<10

CFU = colony forming units; MPN = most probable number
[a] Measured on a dry weight basis
[b] Method detection limit (MDL) was 10 mg/kg

Table II.e.3.2-2 Summary of the Microbiological Product Analysis for 3 Lots of KiwiBerry Powder Concentrate

Specification Parameter	Specification	Lot of KiwiBerry Powder Concentrate		
		FD001-P	SG05-0215-P	SG05-0216-P
Total aerobic count (CFU/g)	≤10,000	≤10	≤10	50
Coliforms (MPN/g)	<3	<3	<3	<3
Escherichia coli (MPN/g)	<3	<3	<3	<3
Salmonella spp.	Negative	Negative	Negative	Negative
Moulds (CFU/g)	≤500	<10	<10	<10

[a] Measured on a dry weight basis
[b] Method detection limit (MDL) was 10 mg/kg

II.f Production Control

KiwiBerry Concentrate, derived from hardy kiwi fruit, meets appropriate food-grade specifications and is manufactured according to current GMP.

II.g Potential Effect on Public Health of Hazardous Substances

No hazardous substances are anticipated to be produced by the production of KiwiBerry Concentrate based on the production and specification controls discussed above.

II.h Potential Effect on Public Health of Hazardous Microorganisms

See Section II.e.3.

III HISTORY OF THE ORGANISM USED AS THE SOURCE OF THE NOVEL FOOD

Based on Commission Recommendation 97/618/EC decision trees, the following questions must be addressed pertaining to the history of the source organism (Commission of the European Communities, 1997):

- "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 GM?"
- "Is the source organism characterized?"
- "Is there information to show that the source organism and/or foods obtained from it are not detrimental to human health?"

We will address each point in turn in this section.

III.a Source of KiwiBerry Concentrate

A. arguta is a dicotyledonous, deciduous, perennial, flowering and fruit-bearing, climbing vine reaching 7 m or more in height and that requires a long growing season (>150 days without frost) (California Rare Fruit Growers, Inc., 1996; USDA, 2005). The flowers of hardy kiwi are white and functionally dioecious, while the stems and leaves are smooth (Dunn, 1911; Nakai, 1933; Li, 1952). Within its natural range, hardy kiwi grows in thickets (Dunn, 1911; Nakai, 1933; Li, 1952). Due to the hardiness of the plant, hardy kiwi can grow in temperatures as low as -23 to -32°C, although it is sometimes susceptible to frost damage (Strik and Cahn, 1996). In addition to its history of cultivation in China, Japan, Korea, and Siberia, hardy kiwi also is now cultivated in the United States, Canada, France, Germany, Italy, and New Zealand (Strik and Cahn, 1996; Ferguson, 1999). The Royal Botanical Gardens at Kew notes that *A. arguta* is grown as "ornamental or fruiting plants" (http://www.rbgkew.org.uk/ksheets/kiwifruit.html).

The hardy kiwi fruit is generally green in colour, ovoid, or oblong in shape (2 to 2.5 cm), unspotted, and fruits are borne in clusters; however, the fruit also may have various tones of reddish skin (Li, 1952; California Rare Fruit Growers, Inc., 1996). Unlike the common green kiwi fruit, which has fuzzy brown skin, the hardy kiwi fruit has an edible smooth skin and is much smaller, resembling a grape (California Rare Fruit Growers, Inc., 1996; Strik and Cahn, 1996; Boyes *et al*., 1997a). The inside of the hardy kiwi fruit looks much like the common green kiwi fruit, with green flesh and black edible seeds, although the hardy kiwi fruit is reported to taste slightly sweeter (California Rare Fruit Growers, Inc., 1996; Boyes *et al*., 1997a; Elstein, 2005). In botanical nomenclature, the fruits are berries (Li, 1952). A picture of hardy kiwi fruit is presented below in Figure III.a-1.

Figure III.a-1 Hardy Kiwi Fruit

Szczepan Marczynski, 2004

III.b Genetically-Modified (GM) Status of *Actinidia arguta*

Actinidia arguta is not a genetically modified organism.

III.c Characterization of *Actinidia arguta*

Hardy kiwi fruit, *A. arguta*, also is commonly referred to as arguta, baby kiwi, cocktail kiwi, or grape kiwi, and belongs to the *Actinidiaceae* family that is indigenous to northern China, Korea, Siberia, and Japan (California Rare Fruit Growers, Inc., 1996). *A. arguta* and the common green kiwi, *A. deliciosa*, descend the same taxonomical route, with each belonging to the genus, *Actinidia* Lindl. The taxonomical designations for *A. arguta* and *A. deliciosa* are outlined in Table III.c-1 below.

Table III.c-1 Taxonomical Designations for Hardy and Green Kiwi Species

Taxonomical Classification	Hardy Kiwi *Actinidia arguta*	Green Kiwi *Actinidia deliciosa*
Kingdom	Plantae	Plantae
Subkingdom	Tracheobionta	Tracheobionta
Superdivision	Spermatophyta	Spermatophyta
Division	Magnoliophyta	Magnoliophyta
Class	Magnoliopsida	Magnoliopsida
Subclass	Dilleniidae	Dilleniidae
Order	Theales	Theales
Family	Actinidiaceae	Actinidiaceae
Genus	*Actinidia* Lindl	*Actinidia* Lindl
Section	Leiocarpe	Stellatae
Species	*Actinidia arguta*	*Actinidia deliciosa*

Adapted from USDA (2005)

The genus *Actinidia* contains more than 75 species (Strik and Cahn, 1996; Boldingh *et al*., 2000; Ferguson, 2005 [unpublished]). The genus has been subdivided into 4 Sections based on morphological features of stems, leaves and fruit, including the structure of leaf hairs, extent of pubescence on stems, leaves and fruit, and the presence or absence of lenticels (spots) on the fruit (Li, 1952; Liang, 1984; Ferguson, 1990a). The Sections containing *A. arguta, A. deliciosa,* and *A. chinensis* are extremely variable. The Section Leiocarpe contains *A. arguta* and other taxa with smooth skinned fruit lacking lenticels, while the Section Stellatae contains *A. chinensis* and *A. deliciosa,* whose stems, leaves and fruit are covered with stellate hairs. The Section designation for *A. arguta* is further divided into Series (*Lamellatae* C.F. Liang), with a full species designation of *Actinidia arguta* (Sieb. and Zucc.) Planch. ex Miq. Identified scientific synonyms for *A. arguta* include the following: *Trochostigma arguta*, *Trochostigma rufa*, *Actinidia rufa*, *Actinidia cordifolia*, *Actinidia platyphylla*, *Actinidia arguta var. rufa*, *Actinidia callosa var. rufa*, and *Actinidia giraldii* (Mansfeld, 2001).

While these divisions are generally accepted, it also has been noted that there is a high degree of morphological variability within each species (Ferguson, 1990b). Some of the intraspecies variability may be due to natural hybridization between species (Huang *et al.*, 2002). In other cases variability may result from multiple ploidy levels. *A. arguta,* for

example, has been found in diploid (2n=58), tetraploid (2n=116) and hexaploid (2n=174) forms (Ferguson *et al.*, 1996), and displays a high degree of morphological variability, and may still be differentiating or undergoing speciation (Huang *et al.*, 2002). The common kiwi fruit, *A. deliciosa*, is hexaploid, whereas the closely related *A. chinensis* is diploid.

Compositional studies have generally confirmed the placement of individual species within the different Sections. For example, *A. arguta* has been distinguished from *A. deliciosa* based on leaf flavonoids (Webby *et al.*, 1994), and relative proportions of common fruit sugars and organic acids (Boyes *et al.*, 1997b; Klages *et al.*, 1998; Boldingh *et al.*, 2000). Such studies relied on few individual plants of each species due to the relative paucity of varieties under cultivation and available for study. Extensive compositional data are not available for *A. arguta* representing all 3 ploidy levels. Compositional data from New Zealand (Boyes *et al.*, 1997b) differ significantly from that published in China (Zhang *et al.*, 1992), particularly in regard to sugar composition. Virtually all of the information available on *A. deliciosa* is derived from the cultivar "Hayward" (Ferguson, 1990b). Among the cultivars which were studied, the fruit of *A. arguta* tend to be sweeter, firmer and more aromatic that those of *A. deliciosa* (Ferguson, 1991).

A. deliciosa bears fruit the size of a hen's egg, weighing 80 to 90 g, whereas fruit of *A. arguta* are the size of a grape, weighing 5 to 14 g (Ferguson, 1991; Strik and Cahn, 1996). In spite of these morphological dissimilarities, genetic compatibility between the species is high. Intentional crosses between *A. arguta* and *A. deliciosa* have been successful (Ferguson, 1990b; Ferguson *et al.*, 1996), natural cross-pollination under adjacent cultivation has been documented (Webby *et al.*, 1994), and crosses in the wild are likely due overlap in the species' distributions. *A. arguta* can also be grafted onto *A. deliciosa* (Boyes *et al.*, 1997a,b).

III.d Information on Detrimental Health Effects from the Source Organism and/or Foods Obtained from It

III.d.1 Historical Consumption of Hardy Kiwi Fruit

A. arguta is indigenous to northern China, Japan, Korea, and Siberia, is cultivated in these countries as a fruit, and has a documented history of safe human consumption (Dunn, 1911; Michurin, 1949; Li, 1952; Titlyanov, 1963; Zhang *et al.*, 1992; Anetai *et al.*, 1996; California Rare Fruit Growers, Inc., 1996; Boyes *et al.*, 1997a; Kolbasina, 2000; Mansfeld, 2001). Populations commonly consuming this fruit in Siberia are primarily of European heritage. Hardy kiwi is now cultivated in the United States, Canada, France, Germany, Italy, and New Zealand (Strik and Cahn, 1996; Ferguson, 1999; Strik, 2002), and is used in the production of sauces, wine, jam, and desserts.

See Section X.a.2 for further details on historical consumption of hardy kiwi fruit.

III.d.2 Allergenicity of Kiwi

See Section XIII.c.

IX ANTICIPATED INTAKE/EXTENT OF USE OF THE NOVEL FOOD

Based on Commission Recommendation 97/618/EC decision trees, the following questions must be addressed pertaining to the intake/extent of use of the novel food (Commission of the European Communities, 1997).

- “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?”

We will address each point in turn in this section.

IX.a Intended Use of KiwiBerry Concentrate and Levels of Use in Foods

Efficas intends to market KiwiBerry Concentrate as a food ingredient in a variety of traditional food products in the EU. Food codes representative of each proposed food-use were chosen from the Ministry of Agriculture, Fisheries, and Food (MAFF) food code list associated with each food consumption survey and grouped in food-use categories according to the food type, and main and subsidiary food group classifications detailed within the National Diet and Nutrition Survey (NDNS) reports (UKDA, 1995, 2001; Office for National Statistics, 2005). The serving sizes were derived from Food Portion Sizes by the Food Standards Agency (FSA, 2002). A given food code may not be associated with all three surveys; as with each new survey, the food code list has been updated to reflect the availability of new foods and the discontinuation of certain obsolete codes. The individual proposed food-uses and use-levels of KiwiBerry Concentrate from all proposed food-uses are summarised in Table IX.a-1. The use of KiwiBerry Concentrate in food and beverage products is self-limiting due to its pH and textural properties.

Table IX.a-1 Summary of the Individual Food-Uses and Use-Levels for KiwiBerry Concentrate in the European Union

Food Category	Proposed Food-Use	Use-Levels (g/serving)	Serving Size* (g)	Use-Level (g/100g)
Beverages	Fruit Juice and Vegetable Juice	1.2	250	0.48
	Meal Replacements	1.2	250	0.48
	Soft Drinks, Not Carbonated, Ready-to-Drink (Regular & Low Calorie); Including All Fruit Flavoured Drinks & Iced Teas**	1.2	250	0.48
	Sports, Energy and Isotonic Drinks (Carbonated & Not Carbonated)	1.2	250	0.48
Cereal and Cereal Products	Biscuits	0.6	4 to 30	2 to 15
	Cereal Bars, Energy Bars, and Soy Protein Bars**	1.2	30	4
	Other Breakfast Cereals	0.6	30	2
	Wholegrain and High Fibre Breakfast Cereals	0.6	30 to 180	0.33 to 2
Milk and Milk Products	Dry Milk	0.6	28	2.14
	Meal Replacements	1.2	250	0.48
	Soya Alternative to Milk	0.6	250	0.24
Miscellaneous	Beverages (Dry Weight), Including Cocoa, Ovaltine, Horlicks, Malted Drinks *etc*.	0.6	20 to 30	2 to 3
Sugars, Preserves and Confectionery	Preserves Including Jams, Spreads, Marmalade	1.2	15	8
Vegetables, Potatoes & Savoury Snacks	Crisps and Savoury Snacks	0.6	30	2

*Serving sizes were approximated from the Food Portion Sizes guide (3rd edition) by Food Standards Agency (FSA, 2002).
**No food codes were identified for Iced Tea or Soy Protein Bars in the MAFF food code list; all non-carbonated beverages will be included in assessment.

IX.b Estimated Consumption of KiwiBerry Concentrate from Proposed Food-Uses

The consumption of KiwiBerry Concentrate from all proposed food-uses was estimated using data from the NDNS programme. The NDNS programme itself consists of 4 different surveys targeting specific age groups, which were conducted every 3 years in succession. Separate survey data are available from the U.K. Data Archive (UKDA) for the National Diet and Nutrition Survey: Adults Aged 16 to 64 years collected in 2000-2001 (NDNS 2000-2001) (Office for National Statistics, 2005), the National Diet, Nutrition and Dental Survey of Children Aged 1½ to 4½ Years, 1992-1993 (NDNS 1992-1993) (UKDA, 1995), the National Diet and Nutrition Survey: Young People aged 4 to 18 Years (NDNS 1997) (UKDA, 2001), and the National Diet and Nutrition Survey: People Aged 65 Years and Over, 1994-1995. Although all four surveys are available, only the former three were utilised in the generation of estimates in the current intake analysis. When combined, the survey results provide the most current data for use in the evaluation of food-use, food consumption patterns, and nutritional status for individuals residing within the U.K. Weighted 4- or 7-day food records for individuals were selected using a stratified multi-stage random probability design, with sampling of private households throughout Great Britain using postal sectors (UKDA, 1995, 2001) as the primary sampling unit.

NDNS data were collected from individuals as well as households *via* 4- (children, aged 1½ to 4½) or 7-day (young people, aged 4 to 18 and adults, aged 16 to 64) weighted dietary intake records throughout all 4 seasons of the year (4 fieldwork waves of 3 months duration), in order to address variability in eating behaviours due to seasonality. Dietary data were recorded by survey respondents or by parents or guardians in the case of the children's survey for the duration of the survey period. NDNS 2000-2001 contains 7-day weighed dietary records for more than 1,724 individuals aged 16 to 64, while, NDNS 1992-1993 contributes 4-day data from an additional 1,592 children 1½ to 4½ years of age. NDNS 1997 adds 7-day records for approximately 1,700 youth aged 4 to 18 (UKDA, 1995, 2001; Office for National Statistics, 2005). Initial postal questionnaires and interviews were employed to identify eligible children, youth, or adults, respectively, for the surveys. Overall, response rates of 93%, 92%, and 73% were achieved; the maximum response rate (individuals agreeing to the initial dietary interview) from the eligible sample selected for participation in the survey were, 88%, 80%, and 61%, respectively, while only 81%, 64%, and 47% of surveyed individuals completed a full dietary record (Gregory *et al*., 1995; UKDA, 2001; Office for National Statistics, 2005).

The NDNS programme collects physiological, anthropometric, and demographic information from individual survey participants, such as sex, age, measured height and weight (by the interviewer), blood analytes, and other variables useful in characterising consumption in addition to collecting information on the types and quantities of foods being consumed. Further assessment of food intake based on consumption by specific population groups of interest within the total surveyed samples was made possible by the inclusion of this information. In order to compensate for the potential under-representation of intakes from

specific population groups resulting from sample variability due to differential sampling probabilities and differential non-response rates [particularly the lower response rate among males aged 15 to 18 years (UKDA, 2001)], sample weights were developed and incorporated with the youth survey (NDNS, 1997).

Weighting the children's survey data to 7 days facilitated the comparison of adult and youth 7-day dietary survey data to dietary data obtained in the 4-day children's survey. This change was based on the assumption that intake patterns on non-recording weekdays were similar to the intakes on recorded weekdays. The 2 weekend days were not re-weighted. All food and drinks consumed on the 2 recorded weekdays were averaged to obtain a daily intake value, which was then multiplied by 5 to approximate intakes for all weekdays. This data was combined with consumption data from weekend dietary records. The full details of the weighting method employed are provided in Appendix J of the report on the children's diet and nutrition study (Gregory *et al*., 1995).

Estimates for the intake of KiwiBerry Concentrate by the U.K. population were generated and collated by computer, using consumption data from individual dietary records, detailing food items ingested by each survey participant on each of the survey days. Estimates for the daily intake of KiwiBerry Concentrate represent projected 7-day averages for each individual from Days 1 to 7 of NDNS data. The distribution from which mean and percentile intake estimates were produced comprised these average amounts. Mean and percentile estimates were generated using ratio estimation and nonparametric techniques, incorporating survey weights where appropriate (*i.e.*, when using youth data to estimate intakes, as described above) in order to provide representative intakes for specific U.K. population groups. All-person intake refers to the estimated intake of KiwiBerry Concentrate averaged over all individuals surveyed regardless of whether they consumed food products in which KiwiBerry Concentrate is currently proposed for use, and therefore includes "zero" consumers (those who reported no intake of food products proposed to contain KiwiBerry Concentrate during the 7 survey days). All-user intake refers to the estimated intake of KiwiBerry Concentrate by those individuals consuming food products in which the use of KiwiBerry Concentrate is under consideration, hence the 'all-user' designation. Individuals were considered users if they consumed 1 or more food products in which KiwiBerry Concentrate is proposed for use on one of the 7 survey days.

Calculations for the mean, and the 90th, 95th, and 97.5th percentile all-person and all-user intakes, and percent consuming, were performed for each of the following population groups:

children, ages 1½ to 4½;
young people, ages 4 to 10
female teenagers, ages 11 to 18;
male teenagers, ages 11 to 18;
female adults, ages 16 to 64; and
male adults, ages 16 to 64.

Table IX.b-1 summarises the estimated total intake of KiwiBerry Concentrate (g/person/day) from all proposed food-uses in the EU by U.K. population group, while Table IX.b-2 presents the data on a per kilogram body weight basis (mg/kg body weight/day). A complete description of the consumption estimates is provided in Appendix III.

Table IX.b-1 Summary of the Estimated Daily Intake of KiwiBerry Concentrate from All Proposed Food Categories in the U.K. by Population Group (NDNS Data)

Population Group	Age Group (Years)	% User	Actual # of Total Users	All-Person Consumption				All-Users Consumption			
				Mean (g)	Percentile (g)			Mean (g)	Percentile (g)		
					90	95	97.5		90	95	97.5
Children	1½ - 4½	98.8	1,628	3.4	6.1	7.1	8.2	3.4	6.1	7.1	8.2
Young People	4-10	99.6	834	4.8	8.2	9.9	11.9	4.8	8.2	9.9	11.9
Female Teenager	11-18	97.3	434	3.3	6.1	7.6	9.0	3.3	6.1	7.6	9.3
Male Teenager	11-18	99.3	413	4.3	8.5	10.1	12.0	4.3	8.5	10.1	12.0
Female Adult	16-64	91.6	878	2.6	4.9	6.3	8.1	2.7	5.0	6.5	8.5
Male Adult	16-64	91.4	700	2.9	6.4	8.2	10.3	3.1	6.6	8.3	10.4

Table IX.b-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of KiwiBerry Concentrate from All Proposed Food Categories in the U.K. by Population Group (NDNS Data)

Population Group	Age Group (Years)	% User	Actual # of Total Users	All-Person Consumption				All-Users Consumption			
				Mean (mg/kg)	Percentile (mg/kg)			Mean (mg/kg)	Percentile (mg/kg)		
					90	95	97.5		90	95	97.5
Children	1½ - 4½	98.8	1,628	240	415	512	594	243	418	514	598
Young People	4-10	99.6	834	192	339	424	511	193	340	424	511
Female Teenager	11-18	97.3	434	64	129	155	190	65	130	162	195
Male Teenager	11-18	99.3	413	82	162	204	271	83	162	204	271
Female Adult	16-64	91.6	878	37	75	93	135	40	78	95	140
Male Adult	16-64	91.4	700	34	78	102	122	37	81	104	126

The percentage of users was high among all age groups evaluated in the current intake assessment as would be expected for a 7-day survey. Greater than 91.4% of the population groups were estimated to consist of users of those food products in which KiwiBerry Concentrate is currently proposed for use (Table IX.b-1). The population group with the greatest percentage of users was that of young people at 99.6%. Large user percentages within a population group typically lead to similar results for the all-person and all-user consumption estimates, and as a consequence, only the all-user intake results will be discussed in detail.

Young people were determined to have the greatest mean all-user intake of KiwiBerry Concentrate on an absolute basis of the individual population groups, with a value of 4.8 g/person/day, and male teenagers were determined to have the greatest 97.5th percentile all-user intake with a value of 12.0 g/person/day (Table 4.1-1). Female adults had the lowest mean all-user intake at 2.7 g/person/day and children had the lowest 97.5th percentile intake at 8.2 g/person/day.

Conversely, on a body weight basis, children were identified as having the highest intake of any population group, with mean and 97.5th percentile all-user KiwiBerry Concentrate intakes of 243 and 598 mg/kg body weight/day, respectively. Male adults had the lowest mean and 97.5th percentile intakes, at 37 and 126 mg/kg body weight/day (Table IX.b-2), respectively.

Estimates for the mean and 97.5th percentile daily intakes of KiwiBerry Concentrate from each individual food category are summarised in Appendix III, Tables A-1 to A-6 and B-1 to B-6 on a mg/day and mg/kg body weight/day basis, respectively. The U.K. population was identified as being significant consumers of biscuits (61.0 to 91.5% users), crisp and savoury snacks (51.3 to 92.8), and soft drinks, not carbonated, ready-to-drink (regular and low calorie), including all fruit flavoured drinks and iced teas (33.4 to 90.7% users). The U.K. population were not significant consumers of meal replacements (milk and non milk-based), soya alternative to milk, and dry milk, with less than 1% users in most, if not all, population groups.

Tables A-1 to A-6 and B-1 to B-6 also summarise the estimates for the mean all-user intakes of KiwiBerry Concentrate by the individual surveyed populations from each of the individual food-uses on a mg/day and mg/kg body weight/day basis, respectively. The consumption of beverages (dry weight), including cocoa, Ovaltine, Horlicks, and malted drinks made the most significant contribution to the mean and 97.5th percentile all-user intakes of KiwiBerry Concentrate by individual population groups. The highest reliable mean and 97.5th percentile all-user intakes of KiwiBerry Concentrate from beverages were 2,549.33 mg/person/day (30.52 mg/kg body weight/day) in male adults and 9,900.00 mg/person/day (174.60 mg/kg body weight/day) in female adults. The lowest reliable mean and 97.5th percentile all-user intakes of KiwiBerry Concentrate were from crisps and savoury snacks. Female adults had the lowest mean all-user intakes with a value of 220.81 mg/person/day and children had the lowest 97.5th percentile intakes of 711.43 mg/person/day.

On a per kilogram body weight basis, children consuming beverages (dry weight), including cocoa, Ovaltine, Horlicks, malted drinks *etc*. were identified as having the highest mean and 97.5th percentile all-user intakes of KiwiBerry Concentrate of 150.80 and 599.17 mg/kg body weight/day, respectively. The lowest reliable mean and 97.5th percentile all-user intakes of KiwiBerry Concentrate were 3.37 and 11.23 mg/kg body weight/day, respectively, by female adults consuming crisps and savoury snack.

Less than 30 individuals from all population groups reported consuming meal replacements (milk and non milk-based), soya alternative to milk, dry milk, and sports, energy and isotonic drinks formulated with KiwiBerry Concentrate. There were less than 30 individuals in some population groups that reported consuming cereal and energy bars formulated with KiwiBerry Concentrate. In addition, there were less than 160 consumers of preserves including jams, spreads and marmalade, and beverages (dry weight), including cocoa, Ovaltine, Horlicks, malted drinks, *etcetera* in some of the individual population groups. Mean and 97.5th percentile intake estimates based on sample sizes of less than 30 and 160, respectively, may not be considered statistically reliable due to the limited sampling size (LSRO, 1995). As such, the reliability of estimates for the intake of KiwiBerry Concentrate based on the consumption of these foods may be questionable for certain individual population groups. Therefore, these food-uses have not been included when assessing the relative contribution of the individual food-use categories to high-level KiwiBerry Concentrate consumption in these specific population groups, as detailed in Sections 4.2-1 and 4.2-2 of Appendix III.

The current consumption estimates likely overestimate the consumption of all population groups considering that all manufacturers are unlikely to use the maximum use level in all permitted food types, and although short-term food consumption databases adequately indicate the consumption of food products that are consumed frequently, they do not necessarily adequately estimate long-term consumption. Additionally, the specified KiwiBerry Concentrate food-uses are not intended to be marketed directly to infants and children (*i.e.*, are not intended to be incorporated in infant formulae/foods or toddler foods), and hence, the actual infant and children consumption of KiwiBerry Concentrate-enriched food products is expected to be limited. Therefore, although an estimate of the consumption of KiwiBerry Concentrate on a body weight basis in children from all-proposed food uses has been included for completeness of the data, it is considered to be a gross over-estimate of the actual expected intake of the ingredient in infants and children from its addition to food.

An average kiwifruit (*Actinidia deliciosa*) weighs 80 to 90 g, and at approximately 80% moisture, contains on the order of 7.2 g dry weight of solids. Thus, the 90th percentile consumption estimate for all-users is comparable to one serving of kiwifruit per day (range 0.7 - 1.18 servings per day), and the 97.5th percentile consumption estimate for all-users is comparable to 1.5 servings of kiwifruit per day (range 1.14 - 1.7 servings per day).

IX.c At Risk Groups

Individuals who are allergic to kiwi fruit may be at risk of having an allergic reaction to KiwiBerry Concentrate; however, the allergens in hardy kiwi fruit are unstable when heated (Alemán *et al.,* 2004; Fiocchi *et al.,* 2004), and due to the method of production of KiwiBerry Concentrate, it is unlikely that individuals with kiwi fruit allergy would have immunoglobulins that would bind specifically to proteins present in KiwiBerry Concentrate and elicit an allergic reaction. Regardless, individuals with kiwi fruit allergy must still be cautious, and, as with other food allergies, individuals with a known kiwi allergy can manage their condition by avoidance of kiwi. Since KiwiBerry Concentrate will be labelled as originating from hardy kiwi fruit, it is anticipated that individuals with kiwi allergy will avoid ingestion of this product. See Section XIII.c for further details.

IX.d Geographical Restrictions

There are no geographical restrictions anticipated within the European Union for the introduction of this product.

IX.e Replacement of Other Foods in the Diet

KiwiBerry Concentrate is not intended to replace any other foods or food ingredients currently marketed in the European Union.

X INFORMATION FROM PREVIOUS HUMAN EXPOSURE TO THE NOVEL FOOD OR ITS SOURCE

Based on the SCF guidelines, the following questions must be answered in the affirmative to ensure sufficient information pertaining to previous human exposure to the novel food:

- “Is there information from previous direct, indirect, intended or unintended human exposure to the novel food or its source which is relevant to the EU situation with respect to production, preparation, population, lifestyles and intakes?”

- “Is there information to demonstrate that exposure to the novel food is unlikely to give rise to mitochondrial, toxicological and/or allergenicity problems?”

We will address each point in turn in this section.

X.a Natural Occurrence of Hardy Kiwi Fruit in the Diet

X.a.1 Geographical Distribution

The genus *Actinidia* is native to eastern Asia, with the centre of development in China (Darrow and Yerkes, 1937; Li, 1952; Ferguson, 1990a). *A. arguta, A. polygama*, and *A. chinensis*, in particular, are noted for their wide distribution in east Asia where they are common plants (Dunn, 1911; Li, 1952; Ferguson, 1990b). The distribution of *A. arguta* extends from Japan through northeastern Asia (Korea, eastern Siberia, and Manchuria), and through much of China (Darrow and Yerkes, 1937; Ferguson, 1991; Mansfeld, 2001).

In northern China and Japan, *A. arguta* is the most abundant *Actinidia* species, and has been an important source of fruit in the human diet (Li, 1952; Anetai *et al.*, 1996). The English botanist Stephen Dunn (Dunn, 1911), who studied, collected and published data on *Actinidia* species in China and served as Superintendent of The Botanical Forestry Department in Hong Kong, wrote the following:

> "The Actinidias hold somewhat the same position in the vegetation of the Far East that the brambles do in this country[1] – that is to say, they provide a large part of the shrubby growth in wood borders and in hedges, in districts in which they abound, climbing over small trees when occasion offers or forming large straggling bushes on the hill-sides."

Similarly, American *Actinidia* taxonomist Li (1952), of the Smithsonian Institution, described the distribution of *Actinidia* in Asian countries by saying "the species are generally common plants in the thickets of the region and occupy fairly broad ranges" and that 'those of wider ranges' include "*A. arguta*". The Japanese taxonomist Nakai (1933) stated "*Actinidia arguta* is found nearly everywhere in Japan". Several species of *Actinidia* were reported to have been cultivated in the northeastern United States as early as the 1930s, some species for ornamental reasons and some, particularly *A. arguta* and *A. chinensis*, for their fruit (Darrow and Yerkes, 1937).

X.a.2 Historical Consumption of Hardy Kiwi Fruit

As previously mentioned, *A. arguta* is indigenous to northern China, Japan, Korea, and Siberia, and also is cultivated in these countries as a fruit, and has a documented history of human consumption (Dunn, 1911; Michurin, 1949; Li, 1952; Titlyanov, 1963; Zhang *et al.*, 1992; Anetai *et al.*, 1996; California Rare Fruit Growers, Inc., 1996; Boyes *et al.*, 1997a; Kolbasina, 2000; Mansfeld, 2001). Hardy kiwi is now cultivated in the United States, Canada, France, Germany, Italy, and New Zealand (Strik and Cahn, 1996; Ferguson, 1999; Strik, 2002), and is used in the production of sauces, wine, jam, and desserts.

[1] Referring to England, the place of publication.

Evidence of *Actinidia* spp. growth and consumption in China and Japan has been reported by Dunn (1911), who stated:

> "The fruits, which in several species have a greenish pulp of pleasant acid taste, somewhat resembling gooseberries, are collected and eaten in many parts of those countries."

Since *A. arguta* is the most abundant of the *Actinidia* species in Japan and northern China (Li, 1952; Anetai *et al*., 1996), then the statement by Dunn (1911) is likely to include *A. arguta*. Dunn (1911) also stated that the fruit and sap of *Actinidia rufa* have been historically consumed as a drink. According to Dunn's description, *A. arguta* is a variety of *A. rufa*, whereas Li (1952) suggested that the name *A. arguta* preceded *A. rufa,* and thus *A. rufa* is a variety of *A. arguta*. Furthermore, Mansfeld (2001) states that *A. rufa* is a synonym for *A. arguta.* In all cases, the species were described as being very morphologically similar. Alternatively, several recent studies reviewed by Huang *et al*. (2002) support the placement of *A. rufa* and *A. arguta* as separate species.

Anetai *et al*. (1996) described the consumption of *A. arguta* and other native food plants by the Ainu people of Japan, particularly prior to the Showa era (pre-1930). Further, much of the crop of *A. arguta* produced in Oregon, USA currently is exported to Japan (Hurst, 2002).

American taxonomist Li (1952) reported the following regarding the food use of *A. arguta* fruit in China:

> "Actinidia is of economic importance because of the fruits. *Actinidia chinensis* and *A. arguta*, well known as Yang-tao in China, have long been used for their edible fruits, which have a greenish pulp of pleasant acid taste. The fruits are collected from wild plants. *Actinidia arguta* is common in northern China while *A. chinensis* is especially common along the Yangtze valley. Recent efforts in introducing these species into cultivation and in improving their products are highly desirable and commendable."

More recently, approximately 2,000 tons of fruit of *Actinidia kolomikta, A. arguta* and *A. polygama* have been harvested annually in China for use as food (Zhang *et al.*, 1992). Zhang *et al*. (1992) reported that the fruit extracts, prepared by boiling dried fruits, are used traditionally to improve digestion and general health.

In Korea, hardy kiwi fruit is described as being consumed as a food and is listed as a fruit in "The Criteria and Standard of General Food" of the *Food Code* of the Korean Food and Drug Administration (Korean FDA, 2002).

As previously mentioned, hardy kiwi also is indigenous to Siberia. Populations commonly consuming this fruit in Siberia are primarily of European heritage. The local populations in Primorskiy Kray, in southeastern Siberia, were harvesting fruit from the wild and also from plantations established prior to 1955, calling the berries of *A. arguta* "big kishmish" (Titlyanov, 1963). The fruit is reportedly eaten fresh, dried or in cooked form, including as

jams, and is also used to make wine (Titlyanov, 1963). Titlyanov (1963) reported that local populations also have used fresh hardy kiwi fruit for preparing fruit gels, compotes, and pie filling, and when dried, the fruits are much like seedless grapes, currants or raisins. Titlyanov (1963) went on to describe *A. arguta* as "fruit-bearing plants of nutritional significance".

Cultivation of *A. arguta* in Siberia dates back to 1930 when the Russian scientist, Michurin, was reported to make selections of *A. arguta* for food use over a period of several decades (Titlyanov, 1963; Mansfeld, 2001). Michurin was reported to have obtained seeds from southeastern Siberia, and in 1949 he wrote, "the large-fruit variety of *A. arguta* has been growing in my nursery for over twenty-five years", and went on to describe 3 newer selections of *A. arguta* with improved quality (Michurin, 1949).

Similarly, the North American cultivation of *A. arguta*, as well as *A. chinesis*, dates back to at least the 1930s, where both species were grown for their fruits (Darrow and Yerkes, 1937). *A. arguta* was cultivated in New England, while *A. chinensis* was cultivated in Washington, DC and California. The kiwi fruits were consumed fresh and/or used for jellies and sauces.

Evidence of more recent dietary exposure to *A. arguta* is well documented. Researchers in New Zealand reported that *A. arguta* is widely grown across the Northern Hemisphere due to its hardiness, with a wide geographic distribution allowing for a variety of physiological characteristics such as colour, size, yield, and harvesting time. The authors also reported that *A. arguta* is highly desirable due to its sweet taste (Boyes *et al*., 1997a). Additionally, numerous agricultural scientists in the U.S. acknowledge that *A. arguta* is cultivated in the U.S. and produces edible fruits. As such, a number of university publications pertaining to hardy kiwi fruit are available, including: the Oregon State University Extension Service publication, "Growing Kiwifruit" (Strik and Cahn, 1996); the Ohio State University Extension Service Publication, "Kiwifruit and Hardy Kiwi" (Strang and Funt, 1993); and the Pennsylvania State University College of Agricultural Sciences publication, "Small Scale Fruit Production" (Penn State, 2001). These publications include information such as how to purchase and grow *A. arguta* for best fruit yields. Furthermore, the United States Department of Agriculture (USDA) Agricultural Research Service (ARS) lists *A. arguta* as a minor crop[2], producing small edible fruit (USDA, 1999), and the ARS is currently investigating *A. arguta* at the National Germplasm Repository (Corvallis, Oregon) as it is considered to be a crop of interest.

X.a.3 Consumption Data for Kiwi Fruit

The green kiwi fruit (*A. deliciosa*) is the most commonly consumed species of kiwi worldwide, with production exceeding one million tons/year (Ferguson, 1999), which is approximately greater than 907,185 tonnes/year. In a European Union Market Survey, compiled in 2004 (EU Market Survey, 2004), consumption of kiwi fruit (species not specified)

[2] Minor crops in the U.S. are grown on fewer than 300,000 acres nationally.

was reported to range from 15.3 thousand tonnes (approximately 2.65 g/ person/day[3]) in the Netherlands in 1999 to 78 thousand tonnes (approximately 3.77 g/person/day[4]) in Italy in 1999 (EU Market Survey, 2004). Overall imports of kiwi fruit to the EU were reported to range from 463,789 tonnes in 2002 to 507,406 tonnes in 2001. In addition, kiwi fruit production in the EU was reported to be 507,000 tonnes in 2003 and 522,000 tonnes in 2001.

In the U.S., The Economic Research Service (ERS) and the USDA estimated the *per capita* consumption of fresh kiwi fruit (species not specified) on a farm-weight basis. For the year 1999, they estimated the total *per capita* consumption of kiwi fruit to be 0.55 lbs, or approximately 680 mg/person/day (USDA, 2004). *Per capita* consumption, however, has been reported to be as high as 0.59 lbs, or approximately 730 mg/person/day, which was reported in 1993 (USDA, 2004).

Despite the documented historical consumption of hardy kiwi fruit, quantitative consumption data have not been identified.

X.b Kiwi Allergenicity

Kiwi fruit allergy was first described in 1981 (Fine, 1981), and since that time, a number of possible primary allergens have been identified in the fruit, ranging in size from 10 to >67 kDa (Pastorello *et al*., 1996, 1998; Möller *et al*., 1997a,b; Fahlbusch *et al*., 1998; Gavrovic-Jankulovic *et al*., 2002). Kiwi fruit allergy presents a wide variety of symptoms ranging from localized oral allergy syndrome (OAS), most commonly characterized by itching and swelling of the lips, mouth, and throat, to more severe anaphylaxis (CFIA, 2000; Lucas *et al*., 2003).

As with other food allergies, individuals with a known kiwi allergy can manage their condition by avoidance of kiwi. Since KiwiBerry Concentrate will be labelled as originating from hardy kiwi fruit, it is anticipated that individuals with kiwi allergy will avoid ingestion of this product. See Section XIII.c for further details.

[3] Calculated using a population of 15,807,641 for the Netherlands in 1999. (http://www.photius.com/wfb1999/netherlands/netherlands_people.html)
[4] Calculated using a population of 56,735,130 for Italy in 1999. (http://www.photius.com/wfb/wfb1999/italy/italy_people.html)

XI NUTRITIONAL INFORMATION ON THE NOVEL FOOD

Based on Commission Recommendation 97/618/EC decision trees the following questions must be addressed pertaining to nutritional information available on the novel food (Commission of the European Communities, 1997):

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

KiwiBerry Concentrate is not intended to replace any foods in the diet; however, it is essentially equivalent to its source, *A. arguta*, and is compositionally similar to the common green kiwi, and therefore, we will address each point in turn in this section.

XI.a Nutritional Equivalence to Existing Foods

XI.a.1 Compositional Comparison of KiwiBerry Concentrate to Kiwi Fruit

Similar to the common green kiwi fruit, which is composed primarily of water (~84%) and contains lesser amounts of sugar (~8%), fibre (~2%), protein, and vitamins (Netherlands Nutrition Centre, 1996), the hardy kiwi fruit also is predominantly water (~80%) (Efficas, Inc., 2005 [personal communication]). On a dry weight basis, fruits of both species predominantly comprise carbohydrate (~80%), with low amounts of protein, fat, and ash (Efficas, Inc., 2005 [personal communication]).

As discussed earlier an average kiwifruit (*Actinidia deliciosa*) weighs 80 to 90 g, and at approximately 80% moisture, contains on the order of 7.2 g dry weight of solids. Thus, the 90th percentile consumption estimate for all-users is comparable to one serving of kiwifruit per day (range 0.7 - 1.18 servings per day), and the 97.5th percentile consumption estimate for all-users is comparable to 1.5 servings of kiwifruit per day (range 1.14 - 1.7 servings per day).

The compositional analyses (proximate) of 7 lots of KiwiBerry Liquid Concentrate and one lot each of *A. arguta* and *A. deliciosa* fruit were conducted by The National Food Laboratory, Inc. (Dublin, CA) using various methods as described above in Sections I.b through I.d. The results of these analyses are presented in Table XI.a.1-1. As evidenced from the analytical results, the composition of KiwiBerry Concentrate remains proportional to that of the fresh *A. arguta* fruit, which is similar to its cousin, the common green kiwi fruit.

Table XI.a.1-1 Proximate Analysis of KiwiBerry Liquid Concentrate in Comparison to *Actinidia arguta* and *Actinidia deliciosa* Fruit*

Parameter	KiwiBerry Liquid Concentrate[a]	*Actinidia arguta*[b]	*Actinidia deliciosa*[c]
Moisture (%)	33.28 ± 6.1	76.82	83.3
Ash (%)	4.64 ± 0.5	3.28	5.27
Protein (%)	5.91 ± 0.58	6.17	6.65
Fat (%)	4.32 ± 2.14	7.38	11.08
Carbohydrate (%)	85.75 ± 2.83	83.18	77.13
Calories (per 100 g)	399.7 ± 12.98	422.78	437.13

* All measures are expressed on a dry weight basis except for moisture.
[a] Mean ± standard deviation of 7 independent lots of manufactured concentrate. Raw material was Oregon-sourced fruit from 2 years harvest.
[b] Fruit was obtained from Oregon. One lot of fruit was tested.
[c] Fruit was obtained from local grocery store. One lot of fruit was tested.

The carbohydrate, organic acid, phenolic, and mineral content of 7 lots of KiwiBerry Liquid Concentrate also were analysed by Medallion Laboratories (Minneapolis, MN), Shuster Laboratories, Inc. (Canton, MA), The National Food Laboratory, Inc. (Dublin, CA) and ChromaDex, Inc. (Boulder, CO). The results of these analyses are presented in Tables

XI.a.1-2 to XI.a.1-5, along with values reported in the published literature and results of analyses of *A. arguta* and *A. deliciosa* fruit.

The total carbohydrate content of the two species is comparable (Efficas, Inc., 2005 [personal communication]), and very early in fruit development, hardy kiwi fruit contains carbohydrate primarily in the form of starch (Klages *et al*., 1998). Carbohydrate levels in the fruit are reported to depend on the time of season (Klages *et al*., 1998; Boldingh *et al*., 2000), and as the kiwi fruit ripens, net starch breakdown results in the accumulation of glucose, fructose, sucrose, and myo-inositol (inositol) (Klages *et al*., 1998). Inositol is the major carbohydrate in hardy kiwi fruit during the first phase of development (*i.e.,* approximately the first 38 days), representing approximately 60% of all sugars, and is greater in *A. arguta* than *A. deliciosa*, with peak levels reported to reach 55 to 60 mg inositol/g dry weight (Klages *et al*., 1998). With increased development and ripening of the fruit, sucrose is reported to become the dominant sugar in hardy kiwi fruit, followed by decreasing levels of fructose, glucose, and inositol, respectively (Klages *et al*., 1998).

A. arguta cultivated in New Zealand is consistently reported to have a greater sucrose:monosaccharide ratio than that of *A. deliciosa* (Boyes *et al.*, 1997b; Klages *et al.*, 1998; Boldingh *et al.*, 2000); however, this was not the case for fruit harvested in China (Zhang *et al.*, 1992). These differences may be varietal, due to growing conditions or ripeness, or a combination of these factors. Alternatively, the reported differences in sucrose:monosaccharide ratio in Chinese *versus* New Zealand samples may be partially due to sample handling, as samples from New Zealand were frozen immediately after harvest to preserve sucrose. Fruit that is stored before being consumed would be expected to have low sucrose content due to the activity of the enzyme invertase, which cleaves sucrose to glucose and fructose.

Table XI.a.1-2	Carbohydrate Components of KiwiBerry Liquid Concentrate in Comparison to Published and Analytical Values for *Actinidia arguta* and *Actinidia deliciosa* Fruit*				
Carbohydrate Component	**KiwiBerry Liquid Concentrate[a] (Mean ± SD)**	***Actinidia arguta***		***Actinidia deliciosa***	
		Published Values[b]	**Analytical Value[c]**	**Published Values[b]**	**Analytical Value[d]**
Carbohydrate Component (g/100 g)					
Sugars	55.06 ± 3.44	23.3 to 46.2	23.99	13.4 to 38.8	52.8
Starch	0.13 ± 0.01	DNR	0.45	DNR	0.52
Fibre	4.88 ± 1.20	DNR	25.02	DNR	13.17
Sugars (g/100 g)					
Fructose	26.71 ± 2.13	5.5 to 8.5	10.05	5 to 16.5	27.0
Glucose	23.06 ± 1.62	5.0 to 8.5	11.09	4.7 to 12.5	24.9
Inositol	4.99 ± 0.42	1.4 to 2.5	2.85	0.8	0.9
Sucrose[e]	0.30 ± 0.35	2.5 to 27.5	0.00	2.7 to 2.8	0.00

* All measures are expressed on a dry weight basis; DNR = Did not research this topic
[a] Mean ± standard deviation of 7 independent lots of manufactured concentrate. Raw material was Oregon-sourced fruit from 2 years harvest.
[b] Literature values reported for ripe fruit at harvest (Zhang *et al*., 1992; Boyes *et al*., 1997b; Klages *et al*., 1998; Boldingh *et al*., 2000). Calculations were made when necessary to standardize units.
[c] Fruit was obtained from Oregon. One lot of fruit was tested.
[d] Fruit was obtained from local grocery store. One lot of fruit was tested.
[e] Reported sucrose content may be influenced by ripeness and length of storage before analysis due to the activity of invertase enzyme in kiwi fruit. No attempt is made to inactivate invertase during the manufacturing of KiwiBerry Concentrate.

Boyes *et al*. (1997b) reported the presence of citric, malic, oxalic, ascorbic, and quinic acids in ripe hardy kiwi fruit, and analytical data of fruit grown in New Zealand, China, and Japan suggest that the organic acid composition of *A. arguta* and *A. deliciosa* are very similar (Zhang *et al.*, 1992; Boyes *et al.*, 1997b; Okamoto and Goto, 2005).

Table XI.a.1-3	Organic Acid Components of KiwiBerry Liquid Concentrate in Comparison to Published and Analytical Values for *Actinidia arguta* and *Actinidia deliciosa* Fruit*				
Organic acid	**KiwiBerry Liquid Concentrate[a] (Mean ± SD)**	***Actinidia arguta***		***Actinidia deliciosa***	
		Published Values[b]	**Analytical Value[c]**	**Published Values[b]**	**Analytical Value[d]**
Citric acid (mg/g)	74.41 ± 8.67	60 to 60.9	35.33	33.9 to 51	38.3
D-Malic acid (mg/g)	15.96 ± 4.02	11.5 to 13.15	22.86	5 to 13	29.4
Quinic acid (mg/g)	37.67 ± 4.85	25.95 to 75.5	21.18	32.5 to 41.8	44.05
Vitamin C (mg/kg)	12.53 ± 20.83	DNR	149.70	DNR	452.50

* All measures are expressed on a dry weight basis; DNR = Did not research this topic.
[a] Mean ± standard deviation of 7 independent lots of manufactured concentrate. Raw material was Oregon-sourced fruit from 2 years harvest.
[b] Literature values reported for ripe fruit were on a wet weight basis (Zhang *et al*., 1992; Boyes *et al*., 1997b; Okamoto and Goto, 2005). These values were multiplied by 5 to estimate dry weight values.
[c] Fruit was obtained from Oregon. One lot of fruit was tested.
[d] Fruit was obtained from local grocery store. One lot of fruit was tested.

Flavonoids detected in KiwiBerry Liquid Concentrate also were detected in the fresh *A. arguta* fruit and/or in *A. deliciosa*. Quercetin levels in individual lots of KiwiBerry Liquid Concentrate ranged from 21.5 to 82.4 mg/kg, and were 36.07 mg/kg and 16.71 mg/kg in the single samples of *A. arguta* and *A. deliciosa* fruit tested, respectively. The levels of isorhamnetin and kaempferol present in KiwiBerry Liquid Concentrate are within range of those identified in fresh *A. deliciosa*. Published data on the flavonoid composition of *A. arguta* fruit were not identified; however, flavonol glycosides, particularly of quercetin, were identified in the leaves of *A. arguta* (Webby, 1991; Webby *et al.*, 1994) and (+)-catechin and (-)-epicatechin were identified in the stems of the fruit (Takano *et al.*, 2003). Analysis of the catechin content of *A. chinensis* Planch was reported to reveal (-)-epicatechin at a level of 4.5 mg/kg ± 1.05 (Arts *et al.*, 2000).

The major anthocyanin in *A. arguta*, cyanidin, was apparently lost during the manufacturing of KiwiBerry Liquid Concentrate, as were most of the other anthocyanins, likely because they are heat labile. Malvidin, which was present in comparable levels in *A. arguta* and *A. deliciosa*, remained during the manufacturing of KiwiBerry Liquid Concentrate. None of the anthocyanins were concentrated during the manufacturing process. The flavonoid and anthocyanin contents of KiwiBerry Liquid Concentrate are also comparable to the levels of these phenolic compounds identified in *A. arguta* and *A. deliciosa.*

A summary of the levels of minerals in KiwiBerry Liquid Concentrate (*i.e.,* sodium, calcium, magnesium, and phosphorus), and the corresponding levels in *A. arguta* and *A. deliciosa* (from analytical and published data) are presented in Table XI.a.1-5. The levels of all of the minerals that were analysed were demonstrated to remain within the range of their respective levels reported in the published literature for *A. arguta* and/or *A. deliciosa*.

Table XI.a.1-5 Mineral Components of KiwiBerry Liquid Concentrate in Comparison to Published and Analytical Values for *Actinidia arguta* and *Actinidia deliciosa* Fruit*

Minerals	KiwiBerry Liquid Concentrate[a] (Mean ± SD)	*Actinidia arguta*		*Actinidia deliciosa*	
		Published Value[b]	Analytical Value[c]	Published Value[b]	Analytical Value[d]
Calcium (mg/kg)	1,267.9 ± 365	2,160	3,623	1,210	2,455
Magnesium (mg/kg)	1,131.9 ± 241	930	906	800	1,018
Phosphorus (mg/kg)	1,993 ± 527	NI	2,066	NI	3,180
Potassium (mg/kg)	21,204 ± 1,062	13,840	11,260	12,710	22,215
Sodium (mg/kg)	338 ± 140	NI	25.02	NI	77.84

* All measures are expressed on a dry weight basis; NI = Not identified
[a] Minerals are expressed as mean + standard deviation of 7 independent lots of manufactured concentrate. Raw material for the KiwiBerry Concentrate was Oregon-sourced fruit from 2 years harvest.
[b] Okamoto and Goto (2005).
[c] Fruit was obtained from Oregon. One lot of fruit was tested.
[d] Fruit was obtained from grocery store. One lot of fruit was tested.

XII MICROBIOLOGICAL INFORMATION ON THE NOVEL FOOD

Based on Commission Recommendation 97/618/EC decision trees the following questions must be addressed pertaining to microbiological information available for the novel food (Commission of the European Communities, 1997):

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

We will address this point in the following section.

XII.a Microbiological Specifications and Analyses for KiwiBerry Concentrate

XII.a.1 Microbiological Specifications for KiwiBerry Concentrate

As the KiwiBerry Concentrates are prepared from a natural source, several microbiological specifications similar to those performed for other food ingredients have been specified to ensure safety of its use in food. Various standard microbial tests appropriate for food ingredients are employed. The microbiological specifications for KiwiBerry Liquid Concentrate and KiwiBerry Powder Concentrate are presented above, in Tables II.e.3.1-1 and II.e.3.1-2, respectively. See Appendix I for details of the analytical methods and product specifications.

XII.a.2 Microbiological Analyses for KiwiBerry Concentrate

Several lots of KiwiBerry Liquid Concentrate and KiwiBerry Powder Concentrate were analysed to verify that the manufacturing process produced a consistent product within the product specifications. A summary of the microbiological product analysis for 7 lots (FD001, SG05-0215A, SG05-0216A, SG05-0217A, SG05-0310-A, SG05-0311-A, and SG05-0312-A) of KiwiBerry Liquid Concentrate is presented above in Table II.e.3.2-1 and demonstrates compliance with final product specifications. Since the KiwiBerry Powder Concentrate is obtained by drying the KiwiBerry Liquid Concentrate using conventional methods, and appropriate storage conditions will be employed, analysis was only performed on 3 lots of KiwiBerry Powder Concentrate (FD001-P, SG05-0215-P, and SG05-216-P). A summary of the microbiological product analysis for KiwiBerry Powder Concentrate is presented above in Table II.e.3.2-2 and demonstrates compliance with final product specifications. See Appendix I for a summary of the analyses, certificates of analysis, and analytical methods.

XIII TOXICOLOGICAL INFORMATION ON THE NOVEL FOOD

Based on Commission Recommendation 97/618/EC decision trees, the following questions must be addressed pertaining to toxicological information available on the novel food (Commission of the European Communities, 1997):

- "Is there a traditional counterpart to the novel food that can be used as a baseline to facilitate the toxicological assessment?"
- "Compared to the traditional counterpart, does the novel food contain any new toxicants or changed levels of existing toxicants?"

OR

- "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?"

We will address each point in turn in this section.

XIII.a Traditional Counterparts as a Baseline for Toxicological Assessment

XIII.a.1 Comparison of KiwiBerry Concentrate to Traditional Counterparts

As mentioned and discussed in detail in Section X, the assessment of the safety of KiwiBerry Concentrate is based on the history of consumption of *A. arguta*, primarily in Asia but also in the northeastern United States, as the fresh fruit, dried or in cooked form, including as jams, jellies, fruit gels, compotes, pie filling, and sauces, and also to make wine (Dunn, 1911; Nakai, 1933; Darrow and Yerkes, 1937; Michurin, 1949; Li, 1952; Titlyanov, 1963; Ferguson, 1990a,b; Zhang *et al.*, 1992; Anetai *et al.*, 1996; California Rare Fruit Growers, Inc., 1996; Ferguson, 1999; Kolbasina, 2000; Mansfeld, 2001; Strik, 2002). The safety of KiwiBerry Concentrate is supported by the proportional composition of the concentrate to the fresh hardy kiwi fruit itself and the relationship and compositional similarity of hardy kiwi fruit to the common green kiwi fruit, which also has enjoyed years of safe consumption by humans. The recognized differences between hardy kiwi and the common green kiwi are the morphological characteristics of each fruit (Ferguson, 1991; Strik and Cahn, 1996) and slight compositional differences in leaf flavonoids and relative proportions of common fruit sugars and organic acids (Webby *et al.*, 1994; Boyes *et al.*, 1997b; Klages *et al.*, 1998; Boldingh *et al.*, 2000); however, in spite of these morphological and slight compositional dissimilarities, genetic compatibility between the species is high and the fruits are qualitatively similar. The relationship of hardy kiwi to the common green kiwi and the compositional similarities between the fresh fruit and the concentrate are discussed in Sections III.c and XI.a. Moreover, the safety of KiwiBerry Concentrate is substantiated by the fact that all components of the ingredient [*i.e.,* mainly carbohydrates (at least 70%), with minor amounts of protein and fat (less than 10% of each), and minimal levels of vitamins, minerals, and flavonoids] are common constituents of the diet and are expected to undergo normal metabolism.

XIII.a.2 Metabolic Fate

As outlined in Sections I.b through I.d, KiwiBerry Concentrate comprises mainly carbohydrate (at least 70%), consisting primarily of fructose, glucose, inositol, and sucrose, with lesser amounts of protein and fat (<10% of each). The metabolism of the major macronutrients present in KiwiBerry Concentrate is discussed below.

The two main sugars present in KiwiBerry Concentrate are glucose and fructose, and inositol and sucrose also are present but at lower levels. Glucose and fructose are monosaccharides, while sucrose is a disaccharide composed of a molecule of glucose and a molecule of fructose (Whitney and Rolfes, 1993a). In the small intestine, sucrose is split into glucose and fructose by the enzyme sucrase, and the monosaccharides are then absorbed into the bloodstream. Fructose is often subsequently converted to glucose in the liver. Glucose is used within the cells of the body for energy, or may be stored as glycogen or fat for future use (Whitney and Rolfes, 1993a). Inositol is a simple isomer of glucose (Levine *et al.*, 1995), and the major dietary form of inositol is inositol hexaphosphate (Raboy, 2003). It

is readily absorbed from the small intestine (Shamsuddin, 1999) and is used in a wide variety of metabolic processes in tissues throughout the body (Raboy, 2003).

As previously mentioned, a small portion of KiwiBerry Concentrate is protein. Following consumption, proteins are denatured in the stomach by acid, and the smaller peptides are cleaved by pepsin. Other enzymes, such as trypsin, chymotrypsin, elastase, and carboxypeptidases, continue to cleave peptide bonds in the small intestine resulting in a mixture of free amino acids and small peptides, which are subsequently transported into mucosal cells by a variety of carrier systems. Within mucosal cells, the small peptides are hydrolysed and free amino acids are then secreted into the portal blood. Amino acids may then be transported to the liver for hepatic use, or may be transported into the systemic circulation for use by peripheral tissues (IOM, 2005).

KiwiBerry Concentrate also contains a small portion of fat, which generally might be present in the diet in the form of triglycerides, free fatty acids, or sterols. Following consumption, very little digestion of fat occurs in the stomach; however, within the small intestine bile acts as an emulsifier and lipase enzymes also serve to digest fat. These enzymes hydrolyze triglycerides releasing fatty acids, glycerol, and phospholipid fragments, which are subsequently absorbed. Glycerol and short- and medium-chain fatty acids are absorbed directly into the bloodstream, while larger lipid fractions, such as phospholipids and cholesterol, are packaged for transport within the intestinal cells. These packages of larger lipid fractions join with small proteins forming lipoproteins, so that they may be transported through the bloodstream. Fat is stored within the body in fat cells or may be used for energy when required (Whitney and Rolfes, 1993b).

The metabolism of the minor components present in KiwiBerry Concentrate, such as phenolics and organic acids, has been well established, and therefore these constituents do not represent any risk to human health.

XIII.b Toxicology Studies

XIII.b.1 Studies of KiwiBerry Concentrate

KiwiBerry Concentrate has been investigated in 5 unpublished sub-chronic toxicity studies in rodents, including a 28-day gavage study in mice (non-GLP), a 28-day oral study in rats (non-GLP), a 76-day gavage study in rats [good laboratory practice (GLP)], a 3-month gavage study in mice (non-GLP), and a 6-month oral study in rats (non-GLP). Additionally, the mutagenic potential of KiwiBerry Concentrate was investigated in the Ames test. The non-GLP studies were conducted by PanGenomics, Inc., a Korean Company. After acquiring rights to the ingredient technology from PanGenomics, Efficas contracted with two independent contract research laboratories to conduct the GLP subchronic study and the Ames test, in order to provide further reassurance. We view the GLP subchronic rat study and the Ames test as pivotal to the safety assessment, and the non-GLP rodent studies as supportive. It should be particularly noted, that since the KiwiBerry Concentrate was not expected to exhibit overt toxicity (based on previous non-GLP studies and the long history of

consumption of the cooked fruit in Asia), in order to be able to detect more subtle adverse impacts on growth, development or behaviour, the GLP compliant subchronic study in rat used a highly vulnerable animal model (juvenile animals dosed by gavage beginning at post-natal day 8) and included additional endpoints such as femur length and a functional observational test battery. The findings were that the no observed adverse effect level (NOAEL) was 2,000 mg/kg body weight, the highest dose tested. Higher doses were not included in the study design since these could imbalance the animal diet, leading to spurious outcomes unrelated to toxicity.

A summary of each of these toxicology studies is provided below in Section XIII.b.1. Moreover, the safety of KiwiBerry Liquid Concentrate was evaluated in a GCP/ICH compliant double blind, randomized, placebo-controlled clinical trial (see Section XIII.b.1.4). A summary table of relevant studies conducted with KiwiBerry Concentrate is presented below (Table XIII.b.1-1).

Table XIII.b.1-1 Studies Conducted with KiwiBerry Concentrate – Studies Conducted to GLP/GCP/ICH				
Study Design and/or Strain (Number)	**Dosage (Oral)**	**Duration**	**Observations Relevant to Safety**	**Reference**
Rat				
Crl:CD rats (20/sex/group) [15/sex/group were necropsied on postnatal Day 85; 5/sex/group on Day 113] GLP	0, 500, 1,000, or 2,000 mg/kg bw/day *via* gavage	76 Days	No significant observations following detailed physical examinations, or effects on food consumption were reported. No significant macroscopic findings were reported in any of the rats that were necropsied on postnatal Day 85 or 113. Absolute counts of monocytes and leukocytes were significantly decreased compared to controls in males dosed with 2,000 mg/kg bw/day on postnatal Day 113. This effect was not observed in females at this dose at postnatal Day 113, nor in males or females at this dose when necropsied on postnatal Day 85. All females treated with KiwiBerry Concentrate were reported to have significantly reduced mean creatinine levels compared to control females on postnatal Day 85. The changes in creatinine were considered incidental by the authors since individual creatinine levels (0.1 to 0.5 mg/dL), as well as the distribution of levels within each group, for all treated females remained very similar to the values for the control group (0.2 to 0.4 mg/dL). Females dosed with 1,000 and 2,000 mg/kg bw/day were reported to have significantly decreased mean absolute and relative uterus/cervix/oviducts weights (relative to brain and final body weight), and also significantly reduced mean kidney weight relative to final body weight compared to controls at postnatal Day 113. Similar effects were not reported in any rat at postnatal Day 85, therefore, these reduced organ weights were considered by the authors to be the result of biological variation. The authors established a NOAEL for KiwiBerry Concentrate of 2,000 mg/kg bw/day, the highest dose tested, when administered orally to rats.	WIL Research Laboratories, 2005 [unpublished]; Beck *et al*. [submitted for publication]
Human				
Randomized, double-blind, placebo-controlled trial in 46 subjects with moderately severe atopic dermatitis GCP/ICH	0 or 600 mg/day	42 Days	No significant changes in haematological, biochemical, or urinary parameters were reported. No serious adverse events were reported by any subject; however, mild side effects were reported in both the KiwiBerry Concentrate and placebo group (12 and 13, respectively). Side effects were deemed by the authors not to be serious and none of the events were considered related to the KiwiBerry Concentrate or placebo. KiwiBerry Concentrate was therefore reported by the authors to be well tolerated.	Mraz *et al*., 2006

AP = alkaline phosphatase; BUN = blood urea nitrogen; GPT = glutamic pyruvic transaminase; LDH = lactose dehydrogenase; NOAEL = no observed adverse effect level
WBC = white blood cells; RBC = red blood cells

Table XIII.b.1-2 Studies Conducted with KiwiBerry Concentrate				
Study Design and/or Strain (Number)	**Dosage (Oral)**	**Duration**	**Observations Relevant to Safety**	**Reference**
Mouse				
Female balb/c mice (5/group)	0 or 150 mg/kg bw/day	28 Days	No reported effects on mortality, clinical signs, body weight gains, or results of haematological and clinical chemistry analyses. No abnormalities reported in histological analysis of the kidney, spleen, thymus, or liver.	PanGenomics Co., Ltd. and Seoul National University, 2002 [unpublished]
5-Week-old balb/c mice (7/sex/group)	0 or 150 mg/kg bw/day	3 Months	No compound-related effects on mortality or clinical signs were reported. Serum concentrations of total protein, bilirubin, and cholesterol, as well as albumin, phosphorus, and chloride were significantly decreased in treatment males compared to controls. Concentrations of phosphorus and sodium were significantly decreased, and creatinine and BUN were significantly increased in females compared to controls. No gross abnormalities reported at necropsy (examined organs not identified).	PanGenomics Co., Ltd. and Seoul National University, 2003a [unpublished]
Rat				
5-Week-old Sprague-Dawley rats (7/sex/group)	0, 100, 300, or 1,000 mg/kg bw/day	28 Days	No effects on mortality, clinical signs, or body weight gain were reported. No histopathological findings in either the heart or the liver (only organs examined) were reported. A number of transient haematological, biochemical, and organ weight changes were reported. Trends towards dose-dependent increases in total bilirubin, GPT, and calcium were reported in males, and also in levels of LDH and calcium in females. Also, males and females dosed with 1,000 mg/kg body weight/day were reported to have significantly increased levels of sodium and AP, and GPT, respectively. Changes in bilirubin, calcium, and sodium were not considered to be toxicologically significant as levels remained within normal reference ranges, nor were the changes in LDH or AP as they are reported to be highly variable in rats (Sharp and La Regina, 1998). WBC, RBC, and platelets were significantly decreased in females at 1,000 mg/kg body weight/day. High dose males and females were reported to have significantly increased brain and lung weights, respectively.	PanGenomics Co., Ltd. and Seoul National University, 2004 [unpublished]
5-Week old Sprague-Dawley rats (7/sex/group)	0 or 300 mg/kg bw/day	6 Months	No reported effects on mortality, clinical signs, or body weight gain. Decreased glucose and increased sodium levels were reported in KiwiBerry Concentrate-treated males compared to control males. In KiwiBerry Concentrate-treated females, the activity of GPT and amylase were increased compared to control females, while concentrations of calcium were decreased. Decreased relative testes weights in males and increased relative liver weights in females were reported compared to controls. No abnormal findings were reported at gross necropsy (organs not specified). The study authors concluded that oral administration of KiwiBerry Concentrate to Sprague-Dawley rats at doses of 300 mg/kg body weight/day for 6 months caused no treatment-related toxicological findings.	PanGenomics Co., Ltd. and Seoul National University, 2003b [unpublished]

Note: These studies, conducted by PanGenomics, gave the code number PG102 to KiwiBerry Concentrate

XIII.b.1.1 Mutagenicity and Genotoxicity Studies

In the Ames assay, the potential mutagenicity of KiwiBerry Concentrate was investigated in *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535, and TA1537 in the presence or absence of metabolic activation (S9) (MDS Pharma Services, 2004 [unpublished]). The concentrations used were 5, 10, 20, 40, 79, 157, 313, 625, 1,250, 2,500, and 5,000 µg/plate both with and without S9. KiwiBerry Concentrate did not induce an increase in revertant colony numbers in any of the concentrations tested, indicating that KiwiBerry Concentrate is non-mutagenic in the Ames assay.

XIII.b.1.2 Sub-chronic Toxicity Studies

A 76-day sub-chronic gavage toxicity study in juvenile rats was sponsored by Efficas, Inc. and conducted by WIL Research Laboratories (Ashland, OH), in accordance with GLP (WIL Research Laboratories, 2005 [unpublished]). Various non-GLP sub-chronic studies of KiwiBerry Concentrate were previously conducted by PanGenomics & SNU (Korea), and include: (1) a 28-day gavage study performed with female balb/c mice; (2) a 3-month gavage study in male and female balb/c mice; (3) a 28-day oral study in male and female Sprague-Dawley rats; and (4) a 6-month oral study in male and female Sprague-Dawley rats (PanGenomics Co., Ltd. and Seoul National University, 2002, 2003a,b, 2004 [unpublished]). A brief review of each of the 5 toxicology studies is provided below.

XIII.b.1.2.1 Studies in the Sprague-Dawley Rat

KiwiBerry Liquid Concentrate was provided to juvenile rats in a 76-day sub-chronic toxicity study, which was conducted in accordance with GLP. Crl:CD rats (20/sex/group) were administered KiwiBerry Concentrate *via* gavage in 1% hydroxypropyl methylcellulose vehicle at a dose of 0, 500, 1,000, or 2,000 mg/kg body weight/day during postnatal Days 8 to 84 (WIL Research Laboratories, 2005 [unpublished]). A separate group of control rats (20/sex) was provided vehicle alone under the same treatment conditions. Fifteen rats/sex/group underwent necropsy one day following the end of the treatment period (*i.e.,* postnatal Day 85), while the remaining 5 rats/sex/group were necropsied following a 4-week non-dosing period (*i.e.,* postnatal Day 113). Developmental parameters (balanopreputial separation and vaginal patency) were assessed during the second week of the study. Functional observation battery (FOB) assessments (*e.g.,* behavioural, sensory, and neuromuscular observations) also were conducted during week 10 of the study period. Haematological and biochemical parameters were evaluated just prior to necropsy in all rats, and included red blood cells (RBC), white blood cells (WBC), platelet, and reticulocyte count, haemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), prothrombin time, activated partial thromboplastin time (APTT), and percent and absolute counts of neutrophils, lymphocytes, monocytes, eosinophils, basophils, as well as large unstained cells. Additionally, the following biochemical parameters were evaluated: albumin, total protein, globulin, total bilirubin, urea nitrogen, creatinine, alkaline phosphatase (AP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyltransferase (GGT), glucose, total cholesterol, calcium, chloride, phosphorus, potassium, and sodium. Absolute organ weights

and organ weights relative to brain or final body weight also were measured at necropsy, and included the adrenal glands, brain, epididymides, kidneys, liver, ovaries, pituitary, prostate, seminal vesicles (with coagulating glands), spleen, testes, thymus, and uterus (including oviducts and cervix). These organs, in addition to the aorta, bone (with marrow), eyes, femur, gastrointestinal tract, heart, lungs, lymph nodes, mammary gland, pancreas, peripheral nerve, salivary glands, skeletal muscle, skin (with mammary gland), spinal cord, thyroid, trachea, urinary bladder, and any other gross lesions, were subsequently macroscopically examined (all rats) at necropsy. The aforementioned organs and tissues also were examined microscopically in 15 rats/sex from the control group and the high-dose group (2,000 mg/kg body weight/day), and also in any rat that died during the experimental period.

One male from each of the control, 500 and 1,000 mg/kg body weight/day dose groups was found dead on postnatal Day 10, 63, and 19, respectively. No clinical findings were reported in any of these rats and no macroscopic findings were identified upon necropsy with the exception of partially collapsed lungs in the male from the 1,000 mg/kg body weight/day group. Furthermore, no cause of death could be determined following microscopic examination of each of the 3 rats. Based on a lack of a dose-response relationship, these deaths were considered by the authors not to be compound-related. An additional rat from the 500 mg/kg body weight/day female dose group was euthanized due to a sexing error (the rat was later determined to be a male). All other male and female rats survived until their scheduled necropsies.

Detailed physical examinations of each rat were conducted throughout the study period, and clinical findings were not significantly different between treatment and control rats. There were no significant differences in any of the measured developmental or FOB parameters. Transient changes in food consumption were reported; however, overall the authors concluded that administration of KiwiBerry Concentrate had no effect on food consumption. Transient changes in body weight parameters also were reported, including a significant decrease in mean body weight gain in females of the 500 mg/kg body weight/day group between postnatal Days 39 to 42; however, this effect was not observed in the higher dose groups and therefore was considered by the authors not to be toxicologically significant. Additionally, females in the mid- and high-dose groups (1,000 and 2,000 mg/kg body weight/day, respectively) were reported to have increased mean body weight gains during the non-dosing period of postnatal Days 91 to 94, although this effect only reached significance in the high-dose group. The group mean body weight gains were increased in these 2 treatment groups by 9.5 and 12.3%, respectively, which was reportedly due to 3 female rats in each group. These 6 rats were reported to weigh 11 to 23% more than the respective group means during the post-treatment period, and 13 to 30% more throughout the dosing period. Consequently, the authors reported that the increased mean body weights observed in the mid- and high-dose groups were due to selection bias.

Absolute counts of monocytes and leukocytes were significantly decreased compared to controls in males dosed with 2,000 mg/kg body weight/day on postnatal Day 113. This effect was not observed in females at this dose at postnatal Day 113, nor in males or females

dosed with 2,000 mg/kg body weight/day and necropsied on postnatal Day 85. Mean cholesterol was significantly reduced in males in the 500 mg/kg body weight/day dose group compared to control rats at postnatal Day 85. This effect was not observed in any other group and was therefore considered by the authors not to be toxicologically significant. All females treated with KiwiBerry Concentrate were reported to have significantly reduced mean creatinine levels compared to control females on postnatal Day 85. The authors reported that the individual creatinine levels for all treated females (0.1 to 0.5 mg/dL), as well as the distribution of levels within each group, remained very similar to the values for the control group (0.2 to 0.4 mg/dL), and therefore the authors considered the statistical significance to be incidental. No other statistically significant differences between KiwiBerry Concentrate-treated rats and control rats were reported in any of the haematological or biochemical parameters tested.

At postnatal Day 85, mean absolute and relative pituitary weights (relative to brain weight) were significantly decreased in males of the 1,000 mg/kg body weight/day dose group compared to control males, and at postnatal Day 113, mean absolute kidney weight was reported to be significantly reduced in males at this dose (1,000 mg/kg body weight/day) compared to controls. Since these effects were not observed in any of the other groups, and thus no dose-response relationship was established, the authors considered these effects on organ weights not to be toxicologically significant. Females dosed with 1,000 and 2,000 mg/kg body weight/day were reported to have significantly decreased mean absolute and relative uterine cervix and oviduct weights (relative to brain and final body weight), and also significantly reduced mean kidney weight relative to final body weight compared to controls at postnatal Day 113. Similar effects were not reported in any rat at postnatal Day 85, therefore these reduced organ weights were considered by the authors to be the result of biological variation.

No significant macroscopic findings were reported in any of the rats that were necropsied on postnatal Day 85 or 113. Following microscopic examination, a single female from the 2,000 mg/kg body weight/day dose group was reported to have a mammary gland adenocarcinoma. The authors reported that such a tumour is extremely rare in a 12-week-old rat, and due to a lack of similar findings in the other female rats at this dose level, the effect was considered by the authors to be incidental. All other microscopic findings observed in rats (both treatment and control) were reported by the authors to be consistent with normal background lesions and were "considered to be spontaneous and/or incidental in nature and unrelated to the test article administration". As such, the authors established a NOAEL for KiwiBerry Concentrate of 2,000 mg/kg body weight/day, the highest dose tested, when administered orally to rats (WIL Research Laboratories, 2005 [unpublished]).

Non-GLP compliant Studies: Five-week-old Sprague-Dawley rats (7/sex/group) were administered KiwiBerry Concentrate orally (exact route not specified) at dose levels of 0, 100, 300, or 1,000 mg/kg body weight/day for a period of 28 days (PanGenomics Co., Ltd. and Seoul National University, 2004 [unpublished]). There were no reported effects on mortality, clinical signs, or body weight gains in either sex at any of the treatment doses, and there were no histopathological findings in either the liver or heart (the only organs

examined). Following an investigation of a number of biochemical and haematological parameters, some changes were reported. Transient but statistically significant increases in blood urea nitrogen (BUN) were reported in males dosed at 100 mg/kg body weight/day and also in glutamic oxaloacetic transaminase (GOT), creatine phosphokinase (CPK), and phosphorous in females dosed with 300 mg/kg body weight/day. Transient decreases in potassium and chloride also were reported in low-dose females (100 mg/kg body weight/day). As all of these effects were transient, they were considered by the authors not to be of toxicological significance. Trends toward dose-dependent increases in total bilirubin, glutamic pyruvic transaminase (GPT), and calcium were reported in males, and also in levels of lactose dehydrogenase (LDH) and calcium in females. Additionally, males and females dosed with 1,000 mg/kg body weight/day were reported to have significantly increased levels of sodium and AP and GPT levels, respectively. The changes in bilirubin, calcium, and sodium were considered not to be toxicologically significant as the levels remained with normal reference ranges [0.0 to 0.64 mg bilirubin/dL blood, 9.1 to 15.1 mg calcium/dL blood, and 142 to 154 mEq sodium/dL blood, respectively (Sharp and La Regina, 1998)], nor were the changes in LDH or AP considered significant, as they are reported to be highly variable in rats and dependent on a number of factors such as haemolysis, animal restraint, and sampling technique (Sharp and La Regina, 1998). A reference range for plasma GPT in rats was not identified in the available literature; however, as there were no gross lesions identified in the liver following histological examination of all KiwiBerry Concentrate-treated rats, the reported effects on GPT or any of the other biochemical parameters tested were considered by the authors not to be of toxicological significance. There were no significant changes in haematological parameters in males dosed with KiwiBerry Concentrate; however, transient changes were reported in the number of neutrophils, lymphocytes, and platelets in the blood of female rats. Additionally, the number of WBC, RBC, and platelets were decreased in females dosed with 1,000 mg/kg body weight/day. These transient and non-dose-dependent changes were considered not to be toxicologically significant by the authors due to a lack of supporting evidence following histological examination. A number of sporadic changes in both absolute and relative organ weight parameters were reported, but according to the authors, they did not appear to be of clinical significance. These changes included increased heart weight in both males and females treated with 100 mg/kg body weight/day, and decreased salivary weights in low-dose males. Females dosed with 100 mg/kg body weight/day also were reported to have increased liver, kidney, spleen, lung, and thymus weights. High dose (1,000 mg/kg body weight/day) males and females were reported to have increased brain and lung weights, respectively. Additionally, a transient increase in hypothalamus weight was reported in the 300 mg/kg body weight male dose group. Additional transient changes in relative organ weights were observed, and included decreased relative heart weight in males dosed with 300 or 1,000 mg/kg body weight/day. The relative weights of liver were decreased in males dosed with 300 mg/kg body weight, but increased in females dosed with 100 mg/kg body weight/day. Additionally, the relative lung weight was increased in mid-dose (300 mg/kg body weight/day) females, while relative brain weight was decreased in females treated with 100 mg/kg body weight/day. Following histological examination of the liver, focal cellular infiltration was observed in one high-dose male and in 1, 4, and 2 females from the low-,

mid-, and high-dose groups, respectively, as well as in 1 male and 2 female control rats; however, the authors reported that this effect was slight and not severe. Similarly, slight focal cellular infiltration of the heart was reported in a single male of each of the KiwiBerry Concentrate treatment groups; however, no dose-response was observed. Focal myocardial degeneration also was reported in a single high-dose treatment rat, which the authors and others (Greaves and Faccini, 1992), have reported is typical of a spontaneous lesion in Sprague-Dawley rats and is not indicative of toxicity. Consequently, the authors stated that no abnormal findings were reported following gross necropsy of the liver and heart (the only organs examined), and concluded that oral administration of KiwiBerry Concentrate to Sprague-Dawley rats at doses of 100, 300, or 1,000 mg/kg body weight/day for 28 days caused no compound-related toxicological findings (PanGenomics Co., Ltd. and Seoul National University, 2004 [unpublished]).

Groups of 7 male and 7 female 5-week-old Sprague-Dawley rats were administered KiwiBerry Concentrate orally (exact route not specified) at dose levels of 0 or 300 mg/kg body weight/day for a period of 6 months (PanGenomics Co., Ltd. and Seoul National University, 2003b [unpublished]). There were no reported effects on mortality, clinical signs, or body weight gains in either sex after 6 months of KiwiBerry Concentrate administration; however, one female treatment rat was reported to die from side effects of anaesthesia. The biochemical analyses revealed decreased glucose and increased sodium levels in treated males compared to control males. In KiwiBerry Concentrate-treated females, the activity of GPT and amylase were increased compared to control females, while the concentrations of calcium were decreased. The numbers of eosinophils were reportedly increased in males at the time of testing of haematological parameters. Organ weight analyses revealed decreased relative testes weights in males and increased relative liver weights in females. No abnormal findings were reported at gross necropsy (organs not specified). The study authors concluded that oral administration of KiwiBerry Concentrate to Sprague-Dawley rats at doses of 300 mg/kg body weight/day for 6 months caused no compound-related toxicological findings (PanGenomics Co., Ltd. and Seoul National University, 2003b [unpublished]).

XIII.b.1.2.2 Studies in the balb/c Mouse

Ten (10) female balb/c mice were administered KiwiBerry Concentrate daily by gavage at dose levels of 0 or 150 mg/kg body weight for a period of 28 days (PanGenomics Co., Ltd. and Seoul National University, 2002 [unpublished]). The haematological parameters assessed included counts of WBC, RBC, lymphocytes, monocytes, neutrophils, eosinophils, basophils, haemoglobin, and platelets. Additionally, clinical chemistry analyses included assessment of the following biological parameters: calcium, sodium, potassium, chloride, phosphorus, glucose, cholesterol, ALT, AST, total protein, bilirubin, albumin, creatinine, BUN, AP, amylase, lipase, lactose dehydrogenase, (LDH), and immunoglobulins E and G (IgE, IgG). There were no reported effects on mortality, clinical signs, body weight gains, or on the results of the haematological and clinical chemistry analyses. No abnormalities were reported in the histological analysis of the kidney, spleen, thymus, and liver of the dosed

animals. The NOAEL could therefore be considered to be 150 mg/kg body weight/day, the only dose tested.

KiwiBerry Concentrate was orally (exact route not specified) administered daily to groups of 5-week-old balb/c mice (7/sex/group) at dose levels of 0 or 150 mg/kg body weight for a period of 3 months (PanGenomics Co., Ltd. and Seoul National University, 2003a [unpublished]). There were no compound-related effects on mortality or clinical signs reported in any of the mice. Body weight gain was reported to be significantly increased in male mice dosed with KiwiBerry Concentrate after 1 and 2 months; however, this was considered by the authors to be normal weight gain. There were no effects on weight gain reported in female mice. A number of biochemical and haematological parameters were assessed, and some changes were reported. The serum concentrations of total protein, albumin, total bilirubin, total cholesterol, phosphorus, and chloride were reportedly decreased in treatment males compared to controls, while concentrations of phosphorus and sodium were decreased in females. Creatinine and BUN also were reportedly increased in females compared to controls. Additionally, organ weight analyses revealed lower kidney, liver, and heart weights in treatment males compared to controls, and increased kidney and liver weights in females treated with KiwiBerry Concentrate compared to controls. On a body weight basis, the relative weights of the heart and liver were decreased in males while the relative liver weight was increased in females compared to control rats. No gross abnormalities were reported at necropsy; however, the examined organs were not identified. The authors concluded that oral administration of KiwiBerry Concentrate to balb/c mice for 3 months caused no compound-related toxicological findings (PanGenomics Co., Ltd. and Seoul National University, 2003a [unpublished]). It may be concluded that the no observed effect level (NOEL) and the NOAEL is 150 mg/kg body weight/day, the only dose tested.

XIII.b.1.3 Other Preclinical Studies of KiwiBerry Concentrate

Park *et al*. (2005) reported that various allergic reactions are thought to be due to imbalances in type-1 and -2 T helper cells (T_H1 and T_H2, respectively), resulting in the overproduction of IgE. Elevated serum IgE is a characteristic of type I hypersensitivity reactions; however, it also is observed in 80% of individuals with atopic dermatitis. Consequently, the authors investigated the potential effect of *A. arguta* extracts on IgE and T_H1 and T_H2 pathways in an *in vitro* model, with the aim of using *A. arguta* as a potential treatment for atopic dermatitis (Park *et al*., 2005). Four different extracts of *A. arguta* were reported to inhibit the production of IgE in LPS-stimulated human B lymphoblastoma (U266B1) cells, which included a total water-soluble extract of *A. arguta* (PG102T), and PG102T extracted with chloroform (PG102C), ethylacetate (PG102E), or n-butanol (PG102B). The PG102E and PG102T extracts were reported to have the strongest inhibitory activities [with 50% inhibitory concentrations (IC_{50}) of 25 and 126 µg/mL, respectively], and therefore were subsequently investigated in an *in vivo* experiment. The *in vivo* experiment was designed to determine the potential effects of PG102T and PG102E on IgE and the production of T_H1 and T_H2 cytokines by splenocytes from ovalbumin-sensitized mice. Mice sensitized to ovalbumin and orally administered distilled water (control), 15 mg PG102T or 1.5 mg PG102E/kg body weight/day for a period of 11 days were killed at the

end of the treatment period and their splenocytes were isolated and incubated in the presence or absence of ovalbumin for a period of 3 days. PG102T and PG102 increased the production of the T_H1 cytokine interleukin (IL)-12 by 1.7 and 2.6-fold, respectively, and also the production of the T_H2 cytokine interferon-gamma (IFN-?) compared to the distilled water control. The PG102T and PG102E extracts were reported to significantly increase the production of the T_H2 cytokines, IL-4, IL-5, and IL-10 by 44, 32, and 44%, respectively, compared to the distilled water control. Furthermore, both PG102 extracts were reported to significantly decrease plasma levels of total immunoglobulin isotypes IgE and IgG2a, although they had no effect on IgG1 or IgG2b. Additionally, the PG102T and PG102E significantly decreased the plasma levels of ovalbumin-specific immunoglobulin isotypes IgE, IgG1, and IgG2a, with no effect on IgG2b. The authors reported that the results suggest PG102T and PG102E may have antiallergenic activity by inhibiting IgE production from cells either directly or indirectly (Park *et al*., 2005).

XIII.b.1.4 Clinical Study of KiwiBerry Concentrate

A single study using KiwiBerry Concentrate was conducted to investigate the potential effect of KiwiBerry Concentrate on dermatitis signs and symptoms in adults with moderately severe atopic dermatitis (Mraz *et al*., 2006). The study was a randomized, double-blind, placebo-controlled trial. Each subject was provided an oral dose of either KiwiBerry Concentrate or placebo (microcrystalline cellulose) at a dose of 600 mg/day for a period of 42 days. A total of 51 subjects enrolled in the study; however, due to the following reasons 5 subjects did not complete the study: subject request, lack of efficacy, protocol violation, non-compliance with study procedures, and one subject discontinued due to elevated laboratory values at Day 1. Additionally, 3 subjects were excluded from the per protocol analysis; therefore, a total of 43 subjects (16 men and 27 women) was included in the analysis. A number of safety parameters were assessed, including full blood chemistry, haematology, and urinalysis which were conducted on Days 1, 14, and 42. There were no statistically significant changes in any of the test parameters measured, which included albumin, AP, ALT, AST, bicarbonate, BUN, calcium, chloride, creatinine, glucose (blood and urine), LDH, phosphorus, potassium, sodium, total protein, uric acid, basophils, eosinophils, hematocrit, haemoglobin, lymphocytes, monocytes, neutrophils, RBCs, WBCs, APTT, prothrombin time, C-reactive protein, total IgE, bilirubin, urinary blood, ketones, nitrite, pH, protein, leukocyte esterase, urobilinogen, and specific gravity. Furthermore, no serious adverse events were reported by either the KiwiBerry Concentrate or placebo group and the incidence and severity of events reported were not significantly different between groups. Twelve (12) side effects were reported in the KiwiBerry Concentrate group and 13 effects were reported in the placebo group (ranging from gastrointestinal effects such as nausea to back pain); however, all side effects were deemed by the authors not to be serious and none of the events were considered related to the KiwiBerry Concentrate or placebo. KiwiBerry Concentrate was therefore reported by the authors to be well tolerated.

XIII.b.2 Other Identified Studies of Kiwi

Upon review of the published literature, few preclinical and clinical studies conducted with hardy kiwi fruit, or kiwi fruit extract in general, were identified; those studies which were identified are summarized in Sections XIII.b.2.1 to XIII.b.2.4 below. The limited amount of data pertaining to kiwi is likely due to the long history of use as a food. A summary table of relevant studies conducted with kiwi is presented below (Table XIII.b.2-1).

Table XIII.b.2-1 Studies Conducted with Kiwi				
Study Design and/or Strain (Number)	**Dosage (Oral)**	**Duration**	**Observations Relevant to Safety**	**Reference**
Rat				
Streptozotocin-induced diabetic rats	2.0 or 2.5% hot water extract *A. arguta* powder [~2,000 or 2,500 mg/kg bw/day] or a 1.0 or 5.0% ethanolic extract of *A. arguta* powder [~1,000 or 5,000 mg/kg bw/day]	5 weeks	Blood glucose was significantly decreased in all rats treated with kiwi compared to baseline; however, there were no significant differences compared to the control group. No adverse effects were reported by the authors.	Han *et al*., 2004 [Abstract only]
Male rats (10/group)	Hydro-alcoholic extract of kiwi (species not specified) at doses of 0, 75, 100, or 150 mg/kg bw/day	50 days	Structural changes were reported in some male reproductive tissues. Some spermatocytes in the testis were reportedly fusiform (dose not specified) following histological examination. This effect was reported to be dose-dependent, and many spermatocytes were reported to be in metaphase in rats dosed with 100 or 150 mg/kg bw/day.	Panjehshahin *et al*., 2003 [Abstract only]
Male rats (10/group)	Hydro-alcoholic extract of *A. chinensis* at doses of 0, 75, 100, or 150 mg/kg bw/day	50 days	Serum testosterone was reported to be significantly decreased in the 150 mg/kg bw/day dose group, and estradiol was significantly decreased in both the 100 and 150 mg/kg bw/day groups compared to the control group and baseline levels. Sperm count and motility were reported to be significantly decreased in the 150 mg/kg bw/day group compared to the control group.	Panjeh-Shahin *et al*., 2005
Human				
Placebo-controlled crossover trial in 6 subjects (3/group)	500 mL homogenized kiwi fruit without skin (species not specified)	Single dose	Kiwi consumption was reported to significantly increase the level of vitamin C in the plasma (peak at 3 hrs after consumption) compared to baseline, and levels returned to baseline within 24 hrs. Kiwi had no significant effect on plasma carotenoids or tocopherols.	Collins *et al*., 2001
Placebo-controlled crossover trial with 25 elite athletes	Kiwi (*A. sinensis*) fruit drink ranging in volume from 500 to 1,200 mL/athlete. Half the volume was consumed 10 minutes prior to training and the rest halfway through the training session.	Single dose (x2), separated by at least 3 days	No significant effect on heart rate, blood pressure, or on the ECG were reported. No side effects were reported by any of the athletes. The authors reported that the kiwi fruit drink supplementation during athletic training was beneficial in maintaining blood glucose and mineral levels, with no significant effect on plasma insulin.	Di *et al*., 1990

Table XIII.b.2-1 Studies Conducted with Kiwi				
Study Design and/or Strain (Number)	**Dosage (Oral)**	**Duration**	**Observations Relevant to Safety**	**Reference**
Randomized crossover trial with 38 elderly subjects (13 male, 25 female)	100 g of kiwi fruit (*A. deliciosa* var. Hayward)/30 kg body weight or regular diet without kiwi fruit	3 weeks	No serious adverse effects were reported. Side effects were reported by 3 participants: 1 case of increased flatulence, 1 case of knee and ankle joint pain, and 1 individual who reported they had "gone off eating kiwi fruit in quantity". Kiwi fruit was reported to significantly enhance all of the self-reported laxative-related parameters investigated.	Rush *et al.*, 2002
Randomized crossover trial in 14 healthy, non-smoking subjects (6 male, 8 female)	1, 2, or 3 whole kiwi fruits (species not specified)/day per dosing period in varying order	3-week dosing periods (x3), separated by a 2-week washout	No adverse effects were reported following consumption of kiwi fruit. Significantly lower levels of DNA breaks in lymphocytes were reported to occur following kiwi fruit consumption (measured *ex vivo via* the comet assay). Kiwi consumption was reported to significantly increase the level of vitamin C in the plasma and lymphocytes compared to baseline after consumption of 2 or 3 kiwi fruit/day, with no significant effect on carotenoids or tocopherols.	Collins *et al.*, 2003
Randomized crossover study in 30 healthy individuals (12 male, 18 female)	Subjects' normal diet supplemented by 2 or 3 whole kiwi fruit (species not specified)	28-day dosing periods (x2), separated by a 2-week washout	Platelet aggregation, induced by both ADP and collagen, was reported to be significantly decreased following consumption of 2 or 3 kiwi fruit compared to baseline; however, the platelet aggregation response returned to baseline levels following the washout periods. Plasma antioxidants and vitamin C levels were significantly increased, and plasma triglycerides were significantly decreased by kiwi fruit consumption (both 2 or 3 kiwis/day) compared to baseline. Kiwi consumption at either level was reported to have no significant effect on total plasma cholesterol, HDL, or LDL. No adverse effects were reported.	Duttaroy and Jorgensen, 2004

ECG = electrocardiogram; DNA = deoxyribonucleic acid; ADP = adenosine diphosphate; HDL = high density lipoprotein; LDL = low-density lipoprotein

XIII.b.2.1 Sub-chronic Toxicity Studies

The effect of 4 different *A. arguta* preparations on blood glucose was investigated in streptozotocin-induced diabetic rats (Han *et al*., 2004). Rats were divided into 4 groups, and were administered either a 2.0 or 2.5% hot water extract of kiwi fruit powder [equivalent to approximately 2,000 or 2,500 mg/kg body weight/day, respectively (U.S. FDA, 1993)], or a 1.0 or 5.0% ethanolic extract of kiwi fruit powder [equivalent to approximately 1,000 or 5,000 mg/kg body weight/day, respectively (U.S. FDA, 1993)] for a period of 5 weeks. An additional group of rats served as the control group. Lipid fractions, glucose, free fatty acids, and ketones were measured in the plasma of all rats both at baseline and after 5 weeks of dosing. At 5 weeks, blood glucose was reported to be significantly decreased in all rats treated with kiwi compared to baseline; however, there were no significant differences compared to the control group. The full findings of this study do not appear to have been published; however, no adverse effects due to kiwi supplementation for a period of 5 weeks were reported in this publication.

XIII.b.2.2 Developmental and Reproductive Toxicity Studies

Panjehshahin *et al*. (2003) published an abstract of a study investigating the effect of a hydro-alcoholic extract of kiwi fruit (species not specified) on the structure of reproductive tissue in male rats (10/group). The authors reported that oral administration of the kiwi extract at doses of 0, 75, 100, or 150 mg/kg body weight/day for 50 days resulted in structural changes in some male reproductive tissues. The testes, ductus deferens, seminal vesicle, prostate, and epididymides of each rat were histologically examined, and revealed that some spermatocytes in the testis had become fusiform. This effect was reported to be dose-dependant, and many spermatocytes were reported to be in metaphase in rats dosed with 100 or 150 mg/kg body weight/day. When stained with acridin orange, many sperm, spermatogonia, and spermatocytes were reported to stain red, indicating denaturing of DNA strands. Some fragmentation of nuclei was reported at 150 mg/kg body weight/day; however, the significance of this effect was not discussed (Panjehshahin *et al*., 2003). The full findings of this study do not appear to have been published, as they could not be identified in the available literature after a significant degree of searching. The toxicological significance of these finding are not clear without thorough examination of the data.

An additional study by the same author investigated the effect of a hydro-alcoholic extract of *A. chinensis* on sperm count and motility and serum levels of estradiol and testosterone (Panjeh-Shahin *et al*., 2005). Male rats (10/group) were provided kiwi extract at a dose of 0 (control), 75, 100, or 150 mg/kg body weight/day for a period of 50 days. Serum testosterone was reported to be significantly decreased in the 150 mg/kg body weight/day dose group, and estradiol was significantly decreased in both the 100 and 150 mg/kg body weight/day groups compared to the control group and baseline levels. No significant changes in estradiol or testosterone levels were reported in the low-dose group (75 mg/kg body weight/day). Additionally, sperm count and motility were reported to be significantly decreased in the 150 mg/kg body weight/day group compared to the control group. Due to similarities in the dosing regime, specimen, and study design, it is unclear whether this study

is in fact the same as the Panjehshahin *et al*. (2003) study, although the study parameters that were reported by the author were not the same. No histological examination was discussed by Panjeh-Shahin *et al*. (2005), and the toxicological significance of these finding are not clear. Furthermore, the findings of these studies are not corroborated by the history of safe consumption of kiwi fruit by both animals and humans, or by the lack of microscopic findings following histological examination of the epididymides, seminal vesicles (with coagulating glands), and testes of male rats provided doses of up to 2,000 mg KiwiBerry Concentrate/kg body weight/day for a period of 76 days (WIL Research Laboratories, 2005 [unpublished]).

XIII.b.2.3 Other Pre-clinical Studies

Traditional mutagenicity/genotoxicity or carcinogenicity studies on kiwi fruit or extract were not identified in the literature; however, the anti-mutagenic potential of kiwi fruit juice and extract has been reported in *S. typhimurium* (Edenharder *et al.*, 1994; Ikken *et al.*, 1999), as were the anti-genotoxic effects of kiwi fruit juice in Chinese hamster lung fibroblasts (Edenharder *et al.*, 2002). Additionally, kiwi fruit was reported to exert strong anti-clastogenic effects against benzo[a]pyrene (Edenharder *et al.*, 1998). Furthermore, extracts of kiwi fruit showed cytotoxic activity against human oral tumour lines (Motohashi *et al.*, 2002), and kiwi fruit juice was reported to provide protection against oxidative DNA damage *ex vivo* and *in vitro* (Collins *et al.*, 2001).

XIII.b.2.4 Clinical Studies

A single clinical study was identified that investigated safety parameters following consumption of kiwi juice by elite Chinese athletes (Di *et al*., 1990). Additional clinical studies designed to investigate parameters such as laxative potential, platelet aggregation, plasma lipids, and antioxidants have been conducted in healthy volunteers and seniors using fresh kiwi fruit (Collins *et al*., 2001, 2003; Rush *et al*., 2002; Duttaroy and Jorgensen, 2004). Kiwi fruit and kiwi juice were well tolerated with no reported side effects. These studies are summarized below.

A single 500 mL dose of homogenized kiwi (species not specified) was provided to 6 healthy volunteers in a placebo-controlled crossover trial (Collins *et al*., 2001). Kiwi consumption was reported to significantly increase the level of vitamin C in the plasma compared to baseline (with peak levels 3 hours after consumption), and levels returned to baseline within 24 hours. Kiwi consumption was reported to have no significant effect on either plasma carotenoids or tocopherols.

The potential effects of Chinese kiwi fruit (*A. sinensis*) juice on elite athletes training in hot environments were investigated in a placebo-controlled, crossover trial (Di *et al*., 1990). Participating athletes were members of the Chinese elite soccer and track teams (16 soccer players, 4 long distance runners, and 5 sprinters), who were between the ages of 18 and 32 and had been training for more than 2 years. The athletes were provided a kiwi fruit drink supplement or placebo (flavoured, coloured water) during 2 training sessions (1.5 to 2.7

hours in duration), which were separated by at least 3 days. Each athlete was reported to consume the same volume of drink in each training session (ranging from 500 to 1,200 mL), where half of the total volume was provided 10 minutes prior to training, and the rest was provided halfway through the training session. A number of parameters were examined by the authors, and these included measurement of heart rate, blood pressure, and an electrocardiogram (ECG), blood glucose, mineral loss, sweat volume and rate, haemoglobin, hemotocrit, oral and skin temperature, and urinary vitamin C. Consumption of the kiwi fruit drink prior to and during training was reported not to have a significant effect on heart rate, blood pressure, or the ECG. Additionally, no side effects were reported by any of the athletes. The authors reported that the kiwi fruit drink supplementation during athletic training was beneficial in maintaining blood glucose and mineral levels, with no significant effect on plasma insulin (Di *et al*., 1990).

Rush *et al*. (2002) investigated the efficacy of kiwi fruit (*A. deliciosa* var. Hayward) as a laxative in a randomized crossover trial in elderly individuals. A total of 38 elderly persons completed the trial (13 male, 25 female), all of whom were >60 years of age and did not have any major bowel problems. Kiwi fruit or no kiwi fruit (*i.e.,* their regular diet) was provided in a 3-week treatment period, with no washout period in between treatments, where each subject received 100 g of kiwi fruit/30 kg of body weight or just their regular diet without kiwi fruit. The laxative potential of kiwi fruit was addressed by self-assessment of parameters such as frequency and ease of defecation, and consistency and volume of stools, based on a preset scale. Each individual served as his or her own control. Side effects were reported by 3 participants and included one case of increased flatulence, one case of knee and ankle joint pain, and one individual who reported they had "gone off eating kiwi fruit in quantity". No serious adverse effects were reported due to consumption of kiwi fruit for 3 weeks, and kiwi fruit was reported to significantly enhance all of the self-reported laxative-related parameters investigated (Rush *et al*., 2002).

A randomized, crossover trial in healthy, non-smoking subjects (6 male, 8 female) was conducted by Collins *et al*. (2003) to determine the effect of kiwi fruit consumption (species not specified) on endogenous oxidation of bases in lymphocyte DNA measured *ex vivo*. Subjects were instructed to maintain their normal diet, and were provided whole kiwi fruit as a supplement for periods of 3 weeks. All subjects were divided into 3 groups and participated in 3 separate 3-week dosing periods, each separated by a 2-week washout period. The 3-week dosing period involved consumption of 1, 2, or 3 whole kiwi fruits/day in varying order. Blood samples were taken from each subject at the start of the study, at the end of each dosing period, and at the end of each washout period. No adverse effects were reported following consumption of kiwi fruit. Using the comet assay to measure the resistance to DNA oxidation *ex vivo*, a significantly lower level of DNA breaks in lymphocytes were reported to occur following kiwi fruit consumption. The authors reported that kiwi fruit consumption increased the level of vitamin C in the plasma and lymphocytes compared to baseline, but had no significant effect on carotenoids or tocopherols.

The effect of kiwi fruit extract on platelet aggregation and plasma lipids and antioxidants was investigated by Duttaroy and Jorgensen (2004) in a randomized, crossover study in 30

healthy individuals (12 male, 18 female). Subjects were instructed to maintain their normal diet and were supplemented with 2 or 3 whole kiwi fruit (species not specified) for a two 28-day dosing period, separated by a 2-week washout period. Blood samples were taken at the start of the study and at the end of each dosing period. Platelet aggregation, induced by both ADP and collagen, was reported to be significantly decreased following consumption of 2 or 3 kiwi fruit compared to baseline; however, the platelet aggregation response returned to baseline levels following the washout periods. Plasma antioxidants and vitamin C levels were significantly increased, and plasma triglycerides were significantly decreased, by kiwi fruit consumption (both 2 or 3 kiwis/day) compared to baseline. Kiwi consumption at either level was reported to have no significant effect on total plasma cholesterol, high-density lipoprotein (HDL), or low-density lipoprotein (LDL). The authors reported that kiwi fruit consumption was well tolerated by all subjects, without any adverse effects (Duttaroy and Jorgensen, 2004).

XIII.c Kiwi Allergenicity

Immunologic-mediated reactions can occur following exposure to allergens from a variety of sources, such as animal dander, chemicals, drugs, foods, fungi, insect stings, and pollen (Bannon, 2004). Such reactions are classified as type I immediate hypersensitivity reactions and are mediated by IgE (Bannon, 2004). Approximately 1 to 2% of adults and up to 8% of children are reported to have food allergies (Sergeant *et al*., 2003), which are elicited by a protein present in the food (Bannon, 2004), and more than 160 foods are reported to induce allergic and hypersensitive reactions mediated by IgE (Romano *et al*., 2000). Kiwi fruit allergy was first described in 1981 (Fine, 1981), and since that time a number of possible primary allergens have been identified in the fruit, ranging in size from 10 to >67 kDa (Möller *et al*., 1997a,b; Pastorello *et al*., 1996, 1998; Fahlbusch *et al*., 1998; Gavrovic-Jankulovic *et al*., 2002). Lucas *et al*. (2003) stated that the discrepancies in dominant allergens [*i.e.,* IgE binding proteins] reported in these studies may be due to use of different experimental procedures or study populations. Of the allergens detected in kiwi fruit, only 3 have been isolated and characterized, namely Act c 1 (30 kDa), Act c 2 (43 kDa), and a thaumatin-like allergen (24 kDa) (Pastorello *et al*., 1998; Gavrovic-Jankulovic *et al*., 2002; Lucas *et al*., 2003). Act c 1 was determined to be a protein called actinidin, the main protein component of kiwi (Pastorello *et al*., 1998). Actinidin has been more specifically characterized as a protease (Boyes *et al*., 1997b).

Allergy to kiwi fruit, primarily *A. deliciosa*, is becoming an increasing common food allergy (Möller *et al*., 1997a,b; Pastorello *et al*., 1998; Bublin *et al*., 2004; Fiocchi *et al*., 2004). Kiwi fruit allergy presents a wide variety of symptoms ranging from localized OAS, most commonly characterized by itching and swelling of the lips, mouth, and throat, to more severe anaphylaxis (CFIA, 2000; Lucas *et al*., 2003). Some individuals with kiwi fruit allergy seem to be also sensitive to pollens, such as birch and grass pollen, timothy, apples, melon, and latex (Möller *et al*., 1997a; Pastorello *et al*., 1998; Rodriguez *et al*., 2000). These cross-reactive allergies are commonly referred to as pollen-food allergy syndrome or latex-food allergy syndrome, in the case of pollen and latex allergy, respectively (Blanco, 2003; Bublin

et al., 2004). Although kiwi fruit allergy is commonly reported in individuals with pollen-fruit or latex-fruit allergy syndrome, it should be noted that some individuals report allergic reactions to kiwi despite a lack of other known allergies (Alemán *et al*., 2004).

Cross-reactivity between green kiwi (*A. deliciosa*) and golden kiwi *(A. chinensis*) also has been reported, as there is evidence that allergens from golden kiwi elicit an allergic reaction in individuals with green kiwi allergy (Bublin *et al*., 2004; Lucas *et al*., 2004). Actinidin, the major allergen in green kiwi, however, was not identified in extracts from golden kiwi (Bublin *et al*., 2004), thus suggesting the allergic reaction was elicited by protein(s) other than actinidin. The potential for cross-reactivity between hardy kiwi (*A. arguta*) allergens and allergens present in green and golden kiwi was evaluated *in vitro* using IgE immunoblots and the direct enzyme linked immunosorbent assay (ELISA) (Chen *et al*., 2006). Samples of fresh hardy kiwi (*i.e.,* non-reduced, unheated sample) and Efficas's KiwiBerry Liquid Concentrate (*i.e.,* reduced, heat-denatured sample) were evaluated using sera from 12 well-documented green kiwi-allergic individuals, and 2 of the individuals were reported the have IgE that binds to protein in fresh hardy kiwi fruit. The protein(s) (~27 kDa) bound by serum IgE, however, did not appear to correspond to the actinidin protein in any of the samples, although this could not be confirmed as the authors did not purify or sequence any of the proteins. Instead, IgE bound to other allergens of various sizes present in hardy kiwi fruit. This evidence indicates that at least some individuals with known allergy to green kiwi fruit are likely to experience allergic reactions to fresh hardy kiwi fruit following consumption. Additionally, with the exception of 1 donor, none of the donors with known green kiwi fruit allergy were reported to have IgE bind to any protein in the KiwiBerry Liquid Concentrate. Allergens in hardy kiwi fruit are heat labile (Alemán *et al.,* 2004; Fiocchi *et al.,* 2004), and the authors reported that "the apparent lack of binding to proteins in processed hardy kiwi fruit concentrate, based on immunoblots, suggests that there is little likelihood that any of these kiwi fruit allergic individuals have IgE that binds specifically to protein present in the processed material"; however, the authors went on to say that the results were only based on *in vitro* data and therefore "caution must be exercised with respect to any broad recommendations regarding the allergenicity of heat-processed hardy kiwi fruit concentrate for the entire population of kiwifruit-allergic consumers" (Chen *et al.,* 2006).

As with other food allergies, individuals with a known kiwi allergy can manage their condition by avoidance of kiwi. Since KiwiBerry Concentrate will be labelled as originating from hardy kiwi fruit, it is anticipated that individuals with kiwi allergy will avoid ingestion of this product.

OVERALL CONCLUSIONS

Efficas intends to market KiwiBerry Concentrate, produced from hardy kiwi fruit, *A. arguta*, as a "novelty fruit" food ingredient in a variety of food products such as beverages, cereal and cereal products, milk and milk products, sugars, preserves and confectionery, and savoury snacks. Hardy kiwi fruit is similar in taste to the common green kiwi fruit, *A. deliciosa*, but is smaller with a fuzzless, smooth skin (Strik and Cahn, 1996). *A. arguta* is indigenous to northern China, Japan, Korea, and Siberia, is cultivated in these countries, as well as France, Germany, Italy, New Zealand, the United States, and Canada, and has a documented history of human consumption (Dunn, 1911; Michurin, 1949; Li, 1952; Titlyanov, 1963; Zhang *et al.*, 1992; Anetai *et al.*, 1996; California Rare Fruit Growers, Inc., 1996; Strik and Cahn, 1996; Boyes *et al.*, 1997a; Ferguson, 1999; Kolbasina, 2000; Mansfeld, 2001; Strik, 2002). Despite the documented historical consumption of hardy kiwi fruit, quantitative consumption data have not been identified.

KiwiBerry Concentrate is manufactured in accordance with current GMP and meets appropriate food-grade specifications. Essentially, KiwiBerry Concentrate is produced by concentrating the hot water soluble components of dried hardy kiwi fruit, with subsequent filtration and evaporative concentration of the crude extract to produce the end product. The aqueous fruit concentrate (KiwiBerry Liquid Concentrate) may then be frozen, freeze-dried, or plated onto a dry inert carrier to produce a free flowing powder (KiwiBerry Powder Concentrate). In order to ensure a consistent product, Efficas has established numerous chemical and microbiological specification parameters for the final preparation, and batch samples are routinely assayed to verify that the set limits are met, ensuring a safe consistent product.

Under the conditions of intended use, the percentage of KiwiBerry Concentrate users was high among all age groups evaluated. Greater than 91.4% of the population groups were estimated to consist of users of those food products in which KiwiBerry Concentrate is proposed for use. The population group with the greatest percentage of users was that of young people at 99.6%. On an absolute basis, young people were determined to have the greatest mean all-user intake of KiwiBerry Concentrate of the individual population groups of 4.8 g/person/day and male teenagers were determined to have the greatest 97.5th percentile all-user intake with a value of 12.0 g/person/day. Conversely, on a body weight basis, children were identified as having the highest intake of any population group, with mean and 97.5th percentile all-user KiwiBerry Concentrate intakes of 243 and 598 mg/kg body weight/day, respectively. Background consumption of kiwi fruit in Italy was calculated to be as high as 3,770 mg/person/day[5], in 1999 (EU Market Survey, 2004), and using an extract ratio of 100 g of fresh hardy kiwi fruit to produce 4 to 5 g of KiwiBerry Concentrate, the background consumption of kiwi fruit in Italy would provide an intake equivalent to approximately 150 to 190 mg KiwiBerry Concentrate/day. An average kiwifruit (*A. deliciosa*) weighs 80 to 90 g, and at approximately 80% moisture, contains on the order of 7.2 g dry

[5] Calculated using a population of 56,735,130 for Italy in 1999 (EU Market Survey, 2004).

weight of solids. Thus, the 90th percentile consumption estimate for all-users is comparable to one serving of kiwifruit per day (range 0.7 - 1.18 servings per day), and the 97.5th percentile consumption estimate for all-users is comparable to 1.5 servings of kiwifruit per day (range 1.14 - 1.7 servings per day).

The assessment of the safety of KiwiBerry Concentrate is based on the proportional composition of the extract to the fresh hardy kiwi fruit itself as well as the relationship and compositional similarity of hardy kiwi fruit to the common green kiwi fruit, which has enjoyed years of safe consumption by humans. The recognized differences between hardy kiwi fruit and the common green kiwi fruit are the morphological characteristics of each fruit (Ferguson, 1991; Strik and Cahn, 1996) and slight compositional differences in leaf flavonoids and relative proportions of common fruit sugars and organic acids (Webby *et al.*, 1994; Boyes *et al.*, 1997b; Klages *et al.*, 1998; Boldingh *et al.*, 2000); however, in spite of these morphological and slight compositional dissimilarities, genetic compatibility between the species is high and the fruits are qualitatively similar. Moreover, the safety of KiwiBerry Concentrate is substantiated by the fact that all components of the ingredient (mainly carbohydrates, with minor amounts of protein and fat, and minimal levels of vitamins, minerals, and flavonoids) are common constituents of the diet and are expected to undergo normal metabolism.

Several unpublished, sub-chronic preclinical toxicity studies have been conducted using KiwiBerry Concentrate (referred to as PG102 by PanGenomics Co, Inc.) and provide supportive evidence for the safety of KiwiBerry Concentrate. A GLP-compliant sub-chronic toxicity study in Crl:CD rats was conducted, where rats were provided doses of up to 2,000 mg of KiwiBerry Concentrate/kg body weight/day for a period of 76 days (WIL Research Laboratories, 2005 [unpublished]). The authors reported there was sufficient scientific evidence to support a NOAEL of 2,000 mg/kg body weight/day for KiwiBerry Concentrate in rats following oral administration. Additionally, the results of 4 other subchronic studies of KiwiBerry Concentrate in rodents, including a 28-day and a 3-month gavage study in mice, and a 28-day and 6-month oral study in rats, indicated no adverse effects following supplementation with the ingredient. Furthermore, KiwiBerry Concentrate was reported to be non-mutagenic in the Ames assay. The limited data available investigating other kiwi species revealed no adverse effects in rats provided hot water kiwi extracts at doses up to 2,500 mg/kg body weight/day or ethanolic kiwi extracts at doses up to 5,000 mg/kg body weight/day for a period of 5 weeks (Han *et al*., 2004). Panjehshahin *et al*. (2003) reported that oral administration of a hydro-alcoholic extract of kiwi (species not specified) at doses of 100 and 150 mg/kg body weight/day for 50 days resulted in structural changes in some male reproductive tissues (*i.e.,* spermatocytes in the testes); however, the full findings of this study do not appear to be published as they could not be identified in the literature, and therefore the toxicological significance of these findings are not clear. An additional study by the same author investigated the effect of a hydro-alcoholic extract of *A. chinensis* on sperm count and motility and serum levels of estradiol and testosterone (Panjeh-Shahin *et al*., 2005). Male rats provided kiwi extract at doses of 0, 75, 100, or 150 mg/kg body weight/day for a period of 50 days were reported to have significantly

decreased serum testosterone (150 mg/kg body weight/day dose group), and significantly decreased serum estradiol (both the 100 and 150 mg/kg body weight/day dose groups) compared to the control group and baseline levels. No significant changes in estradiol or testosterone levels were reported in the low-dose group (75 mg/kg body weight/day). Additionally, sperm count and motility were reported to be significantly decreased in the 150 mg/kg body weight/day group compared to the control group. Due to similarities in the dosing regime, specimen, and study design, it is unclear whether this study is in fact the same as the Panjehshahin *et al*. (2003) study, although the study parameters that were reported by the author were not the same. No histological examination was performed by Panjeh-Shahin *et al*. (2005), and the toxicological significance of these findings are not clear. Furthermore, the findings of these studies are not corroborated by the history of safe consumption of kiwi fruit by both animals and humans, or by the lack of microscopic findings following histological examination of the epididymides, seminal vesicles (with coagulating glands), and testes of male rats provided doses of up to 2,000 mg KiwiBerry Concentrate/kg body weight/day for a period of 76 days (WIL Research Laboratories, 2005 [unpublished]).

A randomized, double blind, placebo-controlled trial demonstrated that consumption of 600 mg of KiwiBerry Concentrate/day for a period of 42 days did not result in any significant changes in blood chemistry, haematology, or urinalysis, or in any adverse effects in adults with moderately severe atopic dermatitis. Furthermore, KiwiBerry Concentrate was reported to be well-tolerated by the subjects. The results of clinical trials using whole kiwi fruit or ingredients derived thereof, indicated that kiwi is well tolerated and without side effects (Di *et al*., 1990; Rush *et al*., 2002; Collins *et al*., 2003; Duttaroy and Jorgensen, 2004). The identified studies ranged in length from a single dose to 28 days and included daily doses of up to 3 whole kiwi fruit or 100 g fresh kiwi fruit/30 kg body weight. Additionally, kiwi fruit juice from *A. chinensis* provided to athletes at a level of 1,200 mL was reported to have no significant effect on heart rate, blood pressure, or their electrocardiogram, and no side effects were reported (Di *et al*., 1990).

Allergy to kiwi is a recognized food allergy, with documented cases of anaphylaxis and allergic and hypersensitivity reactions following kiwi consumption. Additionally, some individuals with kiwi fruit allergy seem to also be sensitive to pollens, such as birch and grass pollen, timothy, apples, melon, and latex, cross-reactivity allergies that are commonly referred to as pollen-food allergy syndrome or latex-food allergy syndrome. Furthermore, cross-reactivity between various species of kiwi also has been reported. There is evidence to suggest that kiwi allergens are heat labile, and thus heated KiwiBerry Liquid Concentrate appears to lack allergenic potential; however, Chen *et al.* (2006) reported that "caution must be exercised with respect to any broad recommendations regarding the allergenicity of heat-processed hardy kiwi fruit concentrate for the entire population of kiwifruit-allergic consumers". Since KiwiBerry Concentrate will be labelled as originating from hardy kiwi fruit, it is anticipated that individuals with kiwi allergy will avoid ingestion of this product.

Overall, the results of pre-clinical and clinical studies of KiwiBerry Concentrate, kiwi fruit, and kiwi extract do not indicate any potential for adverse effects in humans following consumption of KiwiBerry Concentrate under the intended conditions of use. The total body

of published data and information summarised in this report demonstrate that KiwiBerry Concentrate, meeting appropriate food-grade specification and manufactured in accordance with current GMP, would be safe for use as a food ingredient under the conditions of intended use by the history of safe use of hardy and green kiwi fruit and based on the compositional similarity between KiwiBerry Concentrate, hardy kiwi, and green kiwi fruit.

GLOSSARY/LIST OF ABBREVIATIONS

ADP	Adenosine diphosphate
ALT	Alanine aminotransferase
AOAC	Association of Analytical Communities
AP	Alkaline phosphatase
APTT	Activated partial thromboplastin time
ARS	Agricultural Research Service
AST	Aspartate aminotransferase
A_W	Water Activity
BUN	Blood urea nitrogen
CA	California
CFIA	Canadian Food Inspection Agency
CFU	Colony forming units
CO	Colorado
CPK	Creatine phosphokinase
DE	Dry extract
DNA	Deoxyribonucleic acid
EC 258/97	Regulation (EC) No 298/97 of the European Parliament and of the Council of 27th January 1997 concerning novel foods and novel food ingredients
EC 466/2001	Commission Regulation EC No 466/2001
ECG	Electrocardiogram
ELISA	Enzyme linked immunosorbent assay
ERS	Economic Research Service
EU	European Union
FOB	Functional observation battery
FSA	Food Standards Agency
GAP	Good Agricultural Practices
GGT	Gamma glutamyltransferase
GLP	Good Laboratory Practices
GM	Genetically modified
GMP	Good Manufacturing Practices
GOT	Glutamic oxaloacetic transaminase
GPT	Glutamic pyruvic transaminase
HDL	High density lipoprotein
HPLC	High-performance liquid chromatography
IC_{50}	50% Inhibitory Concentrations
IFN-?	Interferon-gamma
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IL	Interleukin
IOM	Institute of Medicine
LDH	Lactose dehydrogenase
LDL	Low density lipoprotein
LPS	Lipopolysaccharide
MA	Massachusetts
MAFF	Ministry of Agriculture, Fisheries, and Food
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
MDL	Method detection limit
MN	Minnesota
NDNS	National Diet and Nutrition Survey
NF	Novel Food

NOAEL	No observed adverse effect level
NOEL	No observed effect level
OAS	Oral allergy syndrome
RACC	Reference Amounts Customarily Consumed Per Eating Occasion
RBC	Red Blood Cells
SCF	Scientific Committee on Food
T_H1	Type-1 T helper cells
T_H2	Type-2 T helper cells
UK	United Kingdom
UKDA	United Kingdom Data Archive
U.S. EPA	United States Environmental Protection Agency
U.S. FDA	United States Food and Drug Administration
U.S.A.	United States of America
USDA	United States Department of Agriculture
USP	United States Pharmacopoeia
WBC	White Blood Cells

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