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ORIGINAL SUBMISSION

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October 16, 2006

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Robert L. Martin, Ph.D.
Deputy Director
Division of Biotechnology and GRAS Notice Review
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway, HFS-255
College Park, MD, 20740

Re: GRAS Notice for KiwiBerry Concentrate

Dear Dr. Martin,

Please find enclosed three copies of a GRAS Notice for KiwiBerry Concentrate, submitted on behalf of Efficas, Inc. The Notice is submitted in accordance with the agency's proposed regulation, proposed 21 CFR 170.36 (62 FR 18938; April 17, 1997; Substances Generally Recognized as Safe (GRAS); the GRAS proposal). This dietary ingredient will be marketed by Efficas, Inc.

Please do not hesitate to contact me if you require additional information.

Sincerely,

[Redacted signature box]

Julianne Lindemann, Ph.D.
Consultant to Efficas
Tel: (510) 669-9496
Tel: (925) 998-1658
FAX: (510) 669-9951

GRAS Exemption Claim

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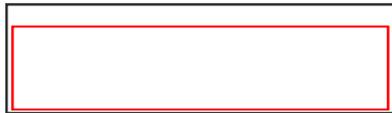
KIWIBERRY CONCENTRATE NOTIFICATION

I GRAS Exemption Claim

A. Claim of Exemption From the Requirement for Premarket Approval Pursuant to Proposed 21 CFR §170.36(c)(1) [62 FR 18938 (U.S. FDA, 1997)]

KiwiBerry Concentrate as defined in the report in Appendix I entitled, "EXPERT PANEL CONSENSUS STATEMENT REGARDING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF KIWIBERRY CONCENTRATE FOR USE IN FOODS," dated August 4, 2006, has been determined to be Generally Recognized as Safe (GRAS) based on scientific procedures, under the conditions of its intended use in food, by experts qualified by scientific training and expertise consistent with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act*. This determination is based on the essential equivalence of KiwiBerry Concentrate to the fresh hardy kiwi, *Actinidia arguta*, which has been consumed historically. The safety of the ingredient is supported by data demonstrating compositional similarity of KiwiBerry Concentrate itself, hardy kiwi and the common green kiwi, the composition of the kiwi concentrate ingredient, and pre-clinical and clinical trials conducted with KiwiBerry Concentrate and other kiwi-derived ingredients and/or kiwi fruit itself, as described in the sections below. Therefore, the use of KiwiBerry Concentrate in food as described below is exempt from the requirement of premarket approval.

Signed,



10/16/06

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

Date

B. Name and Address of Notifier

Dr. Dean Stull
Chief Science Officer
Efficas, Inc.
7007 Winchester Circle, Suite 120
Boulder, CO, USA
80301

C. Common Name of the Notified Substance

The common name of KiwiBerry Concentrate is hardy kiwi concentrate.

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D. Conditions of Intended Use in Food

Efficas, Inc. (Efficas) intends to market KiwiBerry Concentrate as a food ingredient in the United States in a variety of food products including baked goods and mixes, breakfast cereals, chewing gum, dairy product analogs, grain products and pastas, candies, jams and jellies, milk and plant protein products, snack foods, beverages products, and processed fruits and vegetables and their juices (see Table 1).

Table 1 Summary of the Individual Proposed Food-Uses and Use-Levels for KiwiBerry Concentrate in the United States				
Food Category	Proposed Food-Use	Use-Level (mg/RACC)	RACC* (g or mL)	Use-Level (%)
Baked Goods and Baking Mixes	Biscuits	600	55	1.091
	Breads and Rolls	600	50	1.20
	Cookies	600	30 to 40	1.50 to 2.0
	Crackers	600	15 to 30	2.0 to 4.0
	Muffins and Popovers	600	55	1.091
Beverages and Beverage Bases	Iced Teas	600	240	0.250
	Meal Replacements (Not Milk-Based)	600	240	0.250
	Non-alcoholic, Non-carbonated, Non-fruit Beverages	600	240	0.250
	Sports and Isotonic Drinks	600	240	0.250
Breakfast Cereals	Instant and Regular Hot Cereals	600	240	0.250
	Ready-to-Eat Cereals	600	15 to 55	1.091 to 4.0
Chewing Gum	Chewing Gum	600	3	20.0
Dairy Product Analogs	Imitation Milk	600	240	0.250
	Soy Milk	600	240	0.250
Grain Products and Pastas	Cereal and Energy Bars	600	40	1.50
Hard Candy	Hard Candy	600	2 to 15	4.0 to 30.0
Jams and Jellies	Jams, Jellies, and Preserves	600	15	4.0
Milk Products	Milk, Dry and Powdered Mixtures	600	30	2.0
	Milk-Based Meal Replacements	600	240	0.250
Plant Protein Products	Soy Protein Bar	600	40	1.50
Processed Fruits and Fruit Juices	Fruit-Flavored Drinks	600	240	0.250
	Fruit Juices	600	240	0.250
Processed Vegetables and Vegetable Juices	Vegetable Juices	600	240	0.250
Snack Foods	Salty Snacks	600	30	2.0
Soft Candy	Soft Candy	600	40	1.50

* RACC = Reference Amounts Customarily Consumed Per Eating Occasion (21 CFR §101.12). When a range of values is reported for a proposed food-use, particular foods within that food-use may differ with respect to their RACC. (U.S. FDA, 2005a)

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Although not currently on the market, KiwiBerry (Extract) Concentrate may also be sold as a dietary supplement product, with a recommended daily use of 600 to 1,200 mg/day for adults and 300 mg/day for children weighing at least 20 kg or 44 pounds (U.S. FDA, 2005b).

The consumption of KiwiBerry Concentrate from all proposed food uses was estimated using the United States Department of Agriculture (USDA) 1994-1996 Continuing Survey of Food Intakes by Individuals (USDA CSFII 1994-1996), and the 1998 Supplemental Children's Survey (USDA CSFII 1998) (USDA, 2000). On an all-user basis, the mean intake of KiwiBerry Concentrate by the total population from all proposed food-uses was estimated to be 2.39 g/person/day or 47.8 mg/kg body weight/day, with an estimated heavy consumer (90th percentile) all-user intake of 4.40 g/person/day or 105.6 mg/kg body weight/day.

E. Basis for the GRAS Determination

KiwiBerry Concentrate has been determined to be GRAS on the basis of scientific procedures based on its essential equivalence to the fresh hardy kiwi fruit, *Actinidia arguta*, from which it is derived [see Appendix I – **EXPERT PANEL CONSENSUS STATEMENT REGARDING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF KIWIBERRY CONCENTRATE FOR USE IN FOODS**]. The safety of KiwiBerry Concentrate is corroborated by the historical consumption of the hardy kiwi, its composition and similarity to the common green kiwi, *Actinidia deliciosa*, and product-specific and other scientific data.

F. Availability of Information

The data and information that serve as the basis for this GRAS Notification will be sent to the U.S. Food and Drug Administration (U.S. FDA) upon request, or will be available for review and copying at reasonable times at the offices of:

Dr. Julie Lindemann
Consultant to Efficas, Inc.
3260 Blume Dr.
Richmond, CA, USA
94806

Should the U.S. FDA have any questions or additional information requests regarding this notification, Efficas Inc. will supply these data and information.

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II. Detailed Information About the Identity of the Substance

A. Identity

KiwiBerry Concentrate is produced by concentrating the hot water soluble components of dried hardy kiwi fruit, *Actinidia arguta*, 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 tan powdered concentrate (KiwiBerry Powder Concentrate).

Common or Usual Name:	Hardy kiwi concentrate
Chemical Name:	Not applicable
Scientific Name:	<i>Actinidia arguta</i> concentrate
Synonyms:	arguta concentrate; kokuwa concentrate; tara vine concentrate; baby kiwi concentrate; cocktail kiwi concentrate; grape kiwi concentrate
Chemical Abstracts Service (CAS) Number:	KiwiBerry Concentrate has not been assigned a CAS number
Empirical Formula and Formula Weight:	Not defined
Molecular weight:	Not defined
Structural Formula:	Not defined

The hardy kiwi fruit is generally green in color, ovoid or oblong in shape (2 to 2.5 cm), with an inside that looks much like the common fuzzy kiwi (*i.e.*, green flesh and black edible seeds) although the hardy kiwi has an edible smooth skin, is much smaller in size, resembling a grape, and is reported to taste slightly sweeter (Li, 1952; California Rare Fruit Growers, Inc., 1996; Strik and Cahn, 1996; Boyes *et al.*, 1997a; Elstein, 2005). A picture of hardy kiwi fruit is presented in Figure 1.

Figure 1 Hardy Kiwi Fruit (*Actinidia arguta*)



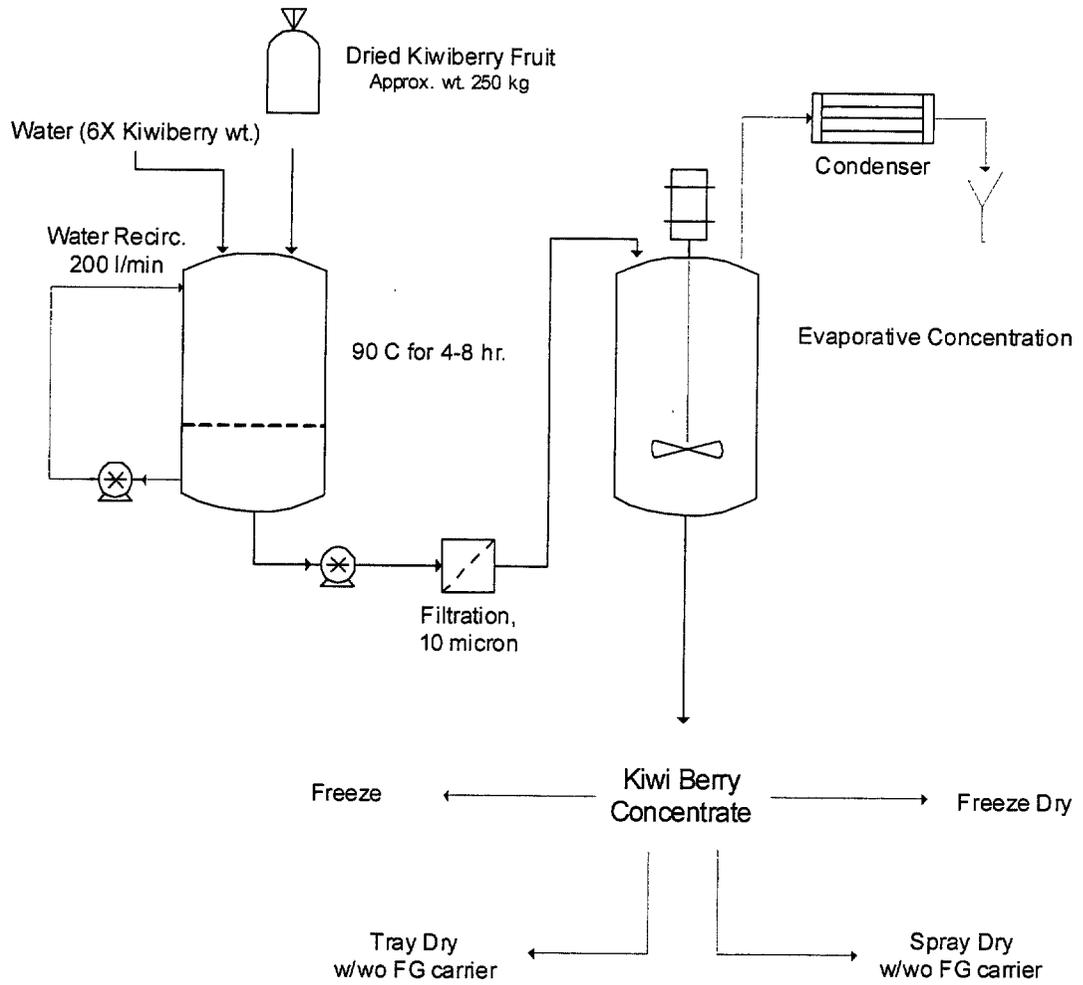
Szczepan Marczyński, 2004

The major components of KiwiBerry Concentrate occur in the hardy kiwi fruit, all of which are present naturally in the human diet *via* consumption of the more common green kiwi fruit, and other fruits and fruit products. These major components include 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 vitamin C, several minerals (calcium, magnesium, phosphorus, potassium, and sodium), organic acids, and phenolic compounds. Processing of *A. arguta* to produce KiwiBerry Concentrate does not disproportionately concentrate any particular component, although fiber levels are greater in fresh fruit than in KiwiBerry Concentrate, most likely due to the fact that most fiber components are not water soluble, and hence the filtration step will remove the insoluble fiber.

B. Method of Manufacture

As mentioned, KiwiBerry Concentrate is produced by concentrating the hot water soluble components of dried hardy kiwi fruit (*A. arguta*) followed by evaporative concentration. Briefly, the hardy kiwi fruit are harvested, sliced, dried, and cooked in hot water, and insoluble components are removed by filtration. Water is the only solvent used in the process. The aqueous fruit concentrate (KiwiBerry Liquid Concentrate) can then be frozen, freeze-dried, or plated onto a dry inert carrier to produce a free flowing powder (KiwiBerry Powder Concentrate). Current Good Manufacturing Practices (cGMP) are employed during all processing operations. For every 100 g of fresh hardy kiwi, 4 to 5 g of KiwiBerry Liquid Concentrate (dry weight) is produced, depending on the water content on the fruit. This is equivalent to a concentration ratio of approximately 25:1. A schematic of the manufacturing process is presented in Figure 2.

Figure 2 KiwiBerry Concentrate Process Overview



FG = food grade

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C. Specifications for Food Grade Material

In order to ensure consistent product(s), Efficas has established numerous chemical and microbiological specification parameters for the final preparations, and representative lots are routinely assayed to ensure compliance with final product chemical, physical, and microbiological specifications. KiwiBerry Concentrate is produced in accordance with cGMP and meets appropriate food-grade specifications, and all processing aids used in the manufacture of the KiwiBerry Powder Concentrate (*i.e.*, microcrystalline cellulose and maltodextrin) are appropriate for food use. The results of studies of the stability of KiwiBerry Concentrate, conducted on the ingredient itself and as a component of a food matrix (*i.e.*, a granola bar), indicated that KiwiBerry Concentrate is very stable at room temperature and under accelerated conditions for up to 3 months. Chemical and microbiological specifications for KiwiBerry Liquid Concentrate and KiwiBerry Powder Concentrate are presented in Table 2.

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Table 2 Chemical and Microbiological Product Specifications for KiwiBerry Concentrate			
Specification Parameter	KiwiBerry Concentrate		Method of Analysis
	Liquid	Powder	
Chemical Parameters			
Moisture	<50%	<6%	AOAC Method 964.22
Carbohydrate	>70% ^a	>75%	By difference ^b
Protein	<10% ^a	<5%	AOAC Method 992.15
Ash	<8% ^a	<4%	AOAC Method 940.262
Fat	<10% ^a	<5%	Sample weight before and after supercritical fluid extraction with CO ₂
Total organic acids	>50 mg/g ^a	>25 mg/g	USP Method 28
Heavy metals	<10 ppm	<10 ppm	USP Method 231
Lead	<1 ppm	<1 ppm	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
<i>Escherichia coli</i>	<3 MPN/g		U.S. FDA BAM, Ch. 4
<i>Salmonella</i> spp.	Negative per 25 g		AOAC Method 996.08
Molds	<500 CFU/g		U.S. FDA BAM, Ch. 18

^a Measured on a dry weight basis

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

AOAC = Association of Analytical Communities; BAM = Bacterial Analytical Manual (Standard Methodologies); CFU = colony forming units; CO₂ = carbon dioxide; ICP-MS = inductively-coupled plasma mass spectrometry; MPN = most probable number; USP = United States Pharmacopoeia.

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III. Self-Limiting Levels of Use

The use of KiwiBerry Concentrate in food and beverage products is self-limiting due to its pH and textural properties.

IV. Basis for GRAS Determination

Pursuant to 21 CFR § 170.30, KiwiBerry Concentrate has been determined to be GRAS on the basis of scientific procedures (U.S. FDA, 2005c). This determination is based on the views of experts who are qualified by scientific training and experience to evaluate the safety of KiwiBerry Concentrate as a component of food.

As mentioned, the major components of KiwiBerry Concentrate occur in the hardy kiwi fruit. These major components include 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 vitamin C, several minerals (calcium, magnesium, phosphorus, potassium, and sodium), organic acids, and phenolic compounds. All of the components of KiwiBerry Concentrate are present naturally in the human diet *via* consumption of the more common green kiwi fruit, and other fruits and fruit products. Processing of *A. arguta* to produce KiwiBerry Concentrate does not disproportionately concentrate any particular component, although fiber levels are greater in fresh fruit than in KiwiBerry Concentrate, most likely due to the fact that most fiber components are not water soluble, and hence the filtration step will remove the insoluble fiber.

Fructose, glucose, inositol, and sucrose are the major sugars present in KiwiBerry Concentrate. Very early in fruit development, hardy kiwi 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 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, followed by decreasing levels of fructose, glucose, and inositol, respectively (Klages *et al.*, 1998).

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 heat labile. The levels of organic acids present in KiwiBerry Concentrate are similar to those present in the fresh fruit.

Comparative analysis of KiwiBerry Concentrate and hardy kiwi fruit to the common green kiwi fruit demonstrates that they are all very similar in composition, and hence, the essential

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equivalence of KiwiBerry Concentrate and its parent fruit, *A. arguta*, to the common green kiwi provide supportive evidence of safety of the ingredient. Additionally, taxonomical evidence indicates that hardy kiwi and fuzzy kiwi are closely related. Similar to the common green kiwi fruit, which is composed primarily of water (~84%) and contains lesser amounts of sugar (~8%), fiber (~2%), protein, and vitamins (Netherlands Nutrition Centre, 1996), the hardy kiwi 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]). Values from the published literature and comparative analyses of the ingredient to samples of the fresh fruits are presented below in Table 3.

Parameter	KiwiBerry Concentrate			<i>Actinidia arguta</i>		<i>Actinidia deliciosa</i>
	Specification		Analytical Value	Published Value ^b	Analytical Value ^c	Analytical Value ^d
	Liquid	Powder	Liquid ^a			
Moisture (%)	<50%	<6%	33.28 ± 6.1	NA	76.82	83.3
Ash (%)	<8%	<4%	4.64 ± 0.5	4.44	3.28	5.27
Protein (%)	<10%	<5%	5.91 ± 0.58	6.85 ± 0.13	6.17	6.65
Fat (%)	<10%	<5%	4.32 ± 2.14	6.62 to 6.69	7.38	11.08
Carbohydrate (%)	>70%	>75%	85.75 ± 2.83	NR	83.18	77.13
Total organic acids (mg/g)	>50	>25	NR	40	NR	NR
Calories (per 100 g)	NA	NA	399.7 ± 12.98	NR	422.78	437.13

* All measures are expressed on a dry weight basis except for moisture.

NA = Not applicable; NR = not reported

^a Mean ± standard deviation of 7 independent lots of manufactured concentrate. Raw material was Oregon-sourced fruit from 2 years harvest.

^b From Zhang *et al.* (1992). Values are the average of 3 measurements.

^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 Range of pure (6.62%) to crude (6.69%) fat.

The total carbohydrate content of the two species is comparable (Efficas, Inc., 2005 [personal communication]). *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; Bolding *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

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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.

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). A summary of the carbohydrate, organic acid, and mineral components of KiwiBerry Concentrate in comparison to published and analytical values for *A. arguta* and *A. deliciosa* is presented in Table 4.

Table 4 Carbohydrate, Organic Acid, and Mineral Components of KiwiBerry Liquid Concentrate in Comparison to Published and Analytical Values for <i>Actinidia arguta</i> and <i>Actinidia deliciosa</i> Fruit*					
Carbohydrate Component	KiwiBerry Liquid Concentrate ^a (Mean ± SD)	<i>Actinidia arguta</i>		<i>Actinidia deliciosa</i>	
		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
Fiber	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	52.8
Glucose	23.06 ± 1.62	5.0 to 8.5	11.09	4.7 to 12.5	0.52
Inositol	4.99 ± 0.42	1.4 to 2.5	2.85	0.8	13.17
Sucrose ^e	0.30 ± 0.35	2.5 to 27.5	0.00	2.7 to 2.8	
Organic Acids					
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 (ppm)	12.53 ± 20.83	DNR	149.70	DNR	452.50
Minerals^f					
Calcium (ppm)	1,267.9 ± 365	2,160	3,623	1,210	2,455
Magnesium (ppm)	1,131.9 ± 241	930	906	800	1,018
Phosphorus (ppm)	1,993 ± 527	NI	2,066	NI	3,180

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Carbohydrate Component	KiwiBerry Liquid Concentrate ^a (Mean ± SD)	<i>Actinidia arguta</i>		<i>Actinidia deliciosa</i>	
		Published Values ^b	Analytical Value ^c	Published Values ^b	Analytical Value ^d
Potassium (ppm)	21,204 ± 1,062	13,840	11,260	12,710	22,215
Sodium (ppm)	338 ± 140	NI	25.02	NI	77.84

* All measures are expressed on a dry weight basis; DNR = Did not research this topic; NI = Not identified

^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 on a wet basis (Zhang *et al.*, 1992; Boyes *et al.*, 1997b; Klages *et al.*, 1998; Bolding *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.

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 ppm, and were 36.07 ppm and 16.71 ppm 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 ppm ± 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 contained in hardy kiwi were observed to concentrate during the KiwiBerry Concentrate manufacturing process. A comparison of the phenolic compounds of KiwiBerry Concentrate to those identified in *A. arguta* and *A. deliciosa* is presented in Table 5.

Phenolic Component	KiwiBerry Liquid Concentrate ^a		<i>Actinidia arguta</i> ^b	<i>Actinidia deliciosa</i> ^c
	Mean \pm SD	Range		
Flavonoids (ppm)				
Quercetin	62.38 \pm 22.57	21.5 to 82.4	36.07	16.71
Isorhamnetin	25.23 \pm 3.22	ND to 31.4	ND	15.87
Kaempferol	23.74 \pm 3.07	ND to 27.7	ND	12.1
Anthocyanins (ppm)				
Cyanidin	1.13 ^d	ND to 1.13	130.07	ND
Delphinidin	0.20 \pm 0.07	ND to 0.32	9.15	0.18
Malvidin	5.9 \pm 5.89	ND to 15.06	11.22	11.68
Pelargonidin	ND	ND	0.69	ND
Peonidin	0.18 \pm 0.14	ND to 0.35	0.99	ND
Catechins	ND	ND	ND	Not tested

* All measures are expressed on a dry weight basis; ND = None detected

^a Mean \pm standard deviation of 7 independent lots of manufactured concentrate with the exception of catechins (1 lot tested). 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.

^d Cyanidin detected in only 1 of 7 lots tested.

V. History of Use of *Actinidia Arguta*

The distribution of *A. arguta* extends from Japan through northeastern Asia (Korea, eastern Siberia, and Manchuria) and much of China (Dunn, 1911; Darrow and Yerkes, 1937; Michurin, 1949; Li, 1952; Titlyanov, 1963; Ferguson, 1990, 1991; Zhang *et al.*, 1992; Anetai *et al.*, 1996; California Rare Fruit Growers, Inc., 1996; Boyes *et al.*, 1997a; Kolbasina, 2000; Mansfeld, 2001). Extensive documentation exists of the consumption of fresh *A. arguta* fruit and as the cooked variation. In northern China and Japan, *A. arguta* has been an important source of fruit in the human diet (Dunn, 1911; Nakai, 1933; Li, 1952; Anetai *et al.*, 1996), and specific evidence of *Actinidia* spp. growth and consumption in China and Japan was reported by Dunn (1911). 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). Regarding the food use of *A. arguta* fruit in China, Li (1952) reported that "*A. arguta*, well known as Yang-tao in China, (has) long been used for (its) edible fruits, which have a greenish pulp of pleasant acid taste." The cultivation and consumption of *A. arguta* in the United States dates back to at least the 1930s, where hardy kiwi and *Actinidia chinensis* species were grown for their fruits (Darrow and Yerkes, 1937). In Siberia, the fruit were harvested from the wild as well as from plantations established before 1955, with reports of use of the fruit being eaten fresh, or in dried or cooked

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form, including as jams, and also to make wine (Titlyanov, 1963; Mansfeld, 2001). 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. In summary, there is a well-documented history of the use of cooked hardy kiwi in various types of foods, as well as consumption of the fresh and dried fruit. The KiwiBerry Concentrate ingredient is most comparable to a jam or jelly-type product since these contain cooked fruit concentrates. The use of *A. arguta* in jams and jellies would likely have yielded a cooked product¹ with a water content of 30 to 40%, which is comparable to the water content of the KiwiBerry Liquid Concentrate. Use of the dried and cooked fruit by individuals in the United States (Darrow and Yerkes, 1937) and by Europeans living in Siberia (Titlyanov, 1963) is well documented. There also is documentation of use of a dried powdered product as a traditional Chinese medicine: Zhang *et al.* (1992) reported that the fruit extracts, prepared by boiling dried fruits, are used traditionally in China to improve digestion and general health. In Korea, hardy kiwi fruit is described as being consumed as a food and used as an herbal medicine (Korean FDA, 2002).

Hardy kiwi is currently cultivated in the United States, Canada, China, France, Germany, and Italy (Zhang *et al.*, 1992; Strik and Cahn, 1996; Ferguson, 1999; Strik, 2002), and researchers in New Zealand reported that *A. arguta* is widely grown across the Northern Hemisphere due to its hardiness, and is highly desirable due to its sweet taste (Boyes *et al.*, 1997a), where *A. arguta* is used in the production of sauces, wine, jam, and desserts. Numerous agricultural scientists acknowledge that *A. arguta* is currently cultivated in the U.S. and produces edible fruits, and various publications pertaining to the cultivation methods of hardy kiwi fruit are available (Strang and Funt, 1993; Strik and Cahn, 1996; Penn State, 2001), and the United States Department of Agriculture (USDA, 2005) Agricultural Research Service (ARS) lists *A. arguta* as a minor crop² and its economic importance as human food.

Due to the similarity in composition between the hardy kiwi and other species of kiwi, it is useful to compare the background consumption of kiwi to intakes of KiwiBerry Concentrate. The *per capita* kiwi consumption in the U.S. was reported to be as high as 0.59 lbs/year in 1993 (USDA, 2004), or approximately 3.56 g/person/day. On a dry weight basis, the composition of KiwiBerry Concentrate to the fresh kiwi fruit is proportional, and hence, the estimated all-person exposure to KiwiBerry Concentrate (2.34 g/day) is within range of the reported *per capita* consumption of kiwi in the U.S.

The safety of KiwiBerry Concentrate is further supported by animal and human data, including several unpublished, subchronic preclinical toxicity studies of KiwiBerry Concentrate,

¹ Water content of multiple jams and jellies adapted from Souci *et al.* (1989).

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

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KIWIBERRY CONCENTRATE NOTIFICATION

mutagenicity data, and a single clinical trial using KiwiBerry Concentrate in adults with moderately severe atopic dermatitis. A limited number of published preclinical studies investigating other kiwi species were identified, and several other clinical studies that investigated the potential effect of kiwi on parameters such as laxative potential, platelet aggregation, plasma lipids, and antioxidants were identified and reviewed. Although some of these studies were not conducted specifically to assess the safety of kiwi, they demonstrated that kiwi was well tolerated and without side effects in humans. Collectively, these studies corroborate the GRAS status of KiwiBerry Concentrate. A summary of the data reviewed by the expert panel is provided in Appendix 1 in a report entitled – **EXPERT PANEL CONSENSUS STATEMENT REGARDING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF KIWIBERRY CONCENTRATE FOR USE IN FOODS**. Copies of selected references that support the GRAS status of KiwiBerry Concentrate based on scientific procedures are provided in Appendix 2.

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February 21, 2005

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Expert Panel Consensus Statement

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EXPERT PANEL CONSENSUS STATEMENT REGARDING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF KIWIBERRY CONCENTRATE FOR USE IN FOODS

July 25, 2006

INTRODUCTION

At the request of Efficas, Inc. (Efficas), an Expert Panel (the "Panel") of independent scientists, qualified by their relevant national and international experience and scientific training to evaluate the safety of food ingredients, was specially convened on December 13, 2005 to conduct a critical and comprehensive evaluation of the available pertinent data and information, and determine whether, under the conditions of intended use as a food ingredient, KiwiBerry Concentrate derived from the hardy kiwi, *Actinidia arguta*, would be Generally Recognized as Safe (GRAS) based on scientific procedures. The Panel consisted of the below-signed qualified scientific experts: Prof. Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University, Medical College of Virginia), Prof. Robert J. Nicolosi, Ph.D. (University of Massachusetts Lowell), and Prof. Stephen L. Taylor, Ph.D. (University of Nebraska). *Curricula vitae* evidencing the Panel members' qualifications for evaluating the safety of food ingredients are provided in Attachment 1.

The Panel, independently and collectively, critically examined a comprehensive package of data and information provided by Efficas. In addition, the Panel evaluated other information deemed appropriate or necessary, including scientific information and data compiled from the literature and other published sources through October 2005 by CANTOX Health Sciences International (CANTOX). The data evaluated by the Panel included information pertaining to the method of manufacture and product specifications, supporting analytical data, intended use-levels in specified food products, consumption estimates for all intended uses, dietary consumption of *A. arguta*, and a comprehensive assessment of the available scientific literature pertaining to the safety of hardy kiwi (*A. arguta*) and other species of kiwi.

Following independent, critical evaluation of such data and information, the Panel unanimously concluded that under the conditions of intended use in traditional foods described herein, KiwiBerry Concentrate derived from hardy kiwi, *A. arguta*, meeting appropriate food-grade specifications and manufactured according to current Good Manufacturing Practice (cGMP), is GRAS based on scientific procedures. The GRAS status of KiwiBerry Concentrate is based on the essential equivalence of the ingredient to the fresh fruit, *A. arguta*, from which it is derived. The safety of KiwiBerry Concentrate is supported by the compositional similarity of the concentrate and *A. arguta* itself to the common green kiwi, *Actinidia deliciosa*, the historical consumption of hardy kiwi, and product-specific and other safety data. A summary of the basis for the Panel's conclusion, excluding confidential data and information, is provided below.

MANUFACTURING AND COMPOSITION

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 dried to produce a powdered concentrate (KiwiBerry Powder Concentrate). For every 100 g of fresh hardy kiwi, 4 to 5 g of KiwiBerry Concentrate solids (dry weight) is produced, depending on the water content of the fruit.

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, several minerals (calcium, magnesium, phosphorus, potassium, and sodium), organic acids, and phenolic compounds. 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 the concentrate product. Anthocyanins also are present in KiwiBerry Concentrate, but most are at levels much lower than identified in the fresh fruit, as they are heat labile. The levels of organic acids present in KiwiBerry Concentrate are similar to those present in the fresh fruit. In addition, the major components of hardy kiwi and KiwiBerry Concentrate itself occur in the more common green kiwi fruit, all of which are present naturally in the human diet *via* consumption of kiwi, other fruits, and other fruit products. Processing of *A. arguta* to produce KiwiBerry Concentrate does not disproportionately concentrate any particular component.

In order to ensure consistent product(s), Efficas has established numerous chemical and microbiological specification parameters for the final preparations, and representative lots are routinely assayed to ensure compliance with final product chemical, physical, and microbiological specifications. KiwiBerry Concentrate is produced in accordance with cGMP and meets appropriate food-grade specifications, and all processing aids used in the manufacture of the KiwiBerry Powder Concentrate (*i.e.*, microcrystalline cellulose and maltodextrin) are appropriate for food use. The results of studies of the stability of KiwiBerry Concentrate, conducted on the ingredient itself and as a component of a food matrix (*i.e.*, a granola bar), indicated that KiwiBerry Concentrate is very stable at room temperature and under accelerated conditions for up to 3 months. Chemical and microbiological specifications for KiwiBerry Liquid Concentrate and KiwiBerry Powder Concentrate are presented in Table 1.

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Table 1 Chemical and Microbiological Product Specifications for KiwiBerry Concentrate			
Specification Parameter	KiwiBerry Concentrate		Method of Analysis
	Liquid	Powder	
Chemical Parameters			
Moisture	<50%	<6%	AOAC Method 964.22
Carbohydrate	>70% ^a	>75%	By difference ^b
Protein	<10% ^a	<5%	AOAC Method 992.15
Ash	<8% ^a	<4%	AOAC Method 940.262
Fat	<10% ^a	<5%	Sample weight before and after supercritical fluid extraction with CO ₂
Total organic acids	>50 mg/g ^a	>25 mg/g	USP Method 28
Heavy metals	<10 ppm	<10 ppm	USP Method 231
Lead	<1 ppm	<1 ppm	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
<i>Escherichia coli</i>	<3 MPN/g		U.S. FDA BAM, Ch. 4
<i>Salmonella</i> spp.	Negative per 25 g		AOAC Method 996.08
Molds	<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; CO₂ = carbon dioxide; ICP-MS = inductively-coupled plasma mass spectrometry; MPN = most probable number; USP = United States Pharmacopoeia

^a Measured on a dry weight basis

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

INTENDED USE AND ESTIMATED EXPOSURE

Efficas intends to market KiwiBerry Concentrate as a food ingredient in the United States in a variety of food products including baked goods and mixes, breakfast cereals, chewing gum, dairy product analogs, grain products and pastas, candies, jams and jellies, milk and plant protein products, snack foods, beverage products, and processed fruits and vegetables and their juices. The individual proposed food-uses and use-levels are summarized below in Table 2. The use of KiwiBerry Concentrate in food and beverage products is self-limiting due to its pH and textural properties.

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Food Category	Proposed Food-Use	Use-Level (mg/RACC)	RACC* (g or mL)	Use-Level (%)
Baked Goods and Baking Mixes	Biscuits	600	55	1.091
	Breads and Rolls	600	50	1.20
	Cookies	600	30 to 40	1.50 to 2.0
	Crackers	600	15 to 30	2.0 to 4.0
	Muffins and Popovers	600	55	1.091
Beverages and Beverage Bases	Iced Teas	600	240	0.250
	Meal Replacements (Not Milk-Based)	600	240	0.250
	Non-alcoholic, Non-carbonated, Non-fruit Beverages	600	240	0.250
	Sports and Isotonic Drinks	600	240	0.250
Breakfast Cereals	Instant and Regular Hot Cereals	600	240	0.250
	Ready-to-Eat Cereals	600	15 to 55	1.091 to 4.0
Chewing Gum	Chewing Gum	600	3	20.0
Dairy Product Analogs	Imitation Milk	600	240	0.250
	Soy Milk	600	240	0.250
Grain Products and Pastas	Cereal and Energy Bars	600	40	1.50
Hard Candy	Hard Candy	600	2 to 15	4.0 to 30.0
Jams and Jellies	Jams, Jellies, and Preserves	600	15	4.0
Milk Products	Milk, Dry and Powdered Mixtures	600	30	2.0
	Milk-Based Meal Replacements	600	240	0.250
Plant Protein Products	Soy Protein Bar	600	40	1.50
Processed Fruits and Fruit Juices	Fruit-Flavored Drinks	600	240	0.250
	Fruit Juices	600	240	0.250
Processed Vegetables and Vegetable Juices	Vegetable Juices	600	240	0.250
Snack Foods	Salty Snacks	600	30	2.0
Soft Candy	Soft Candy	600	40	1.50

* RACC = Reference Amounts Customarily Consumed Per Eating Occasion (21 CFR §101.12). When a range of values is reported for a proposed food-use, particular foods within that food-use may differ with respect to their RACC. (U.S. FDA, 2005)

Using the United States Department of Agriculture (USDA) 1994-1996 Continuing Survey of Food Intakes by Individuals (USDA CSFII 1994-1996) survey (USDA, 2000), under the conditions of intended use, the total population all-user mean intake of KiwiBerry Concentrate was estimated to be 2.39 g/person/day or 47.8 mg/kg body weight/day, with an estimated heavy consumer (90th percentile) all-user intake of 4.40 g/person/day or 105.6 mg/kg body weight/day. The greatest mean and 90th percentile all-user intakes of KiwiBerry Concentrate were estimated to occur in male teenagers, at 3.45 and 6.28 g/person/day, respectively (55.9 and 107.4 mg/kg body weight/day, respectively) (see Tables 3 and 4).

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Population Group	Age Group (Years)	% Users	Actual # of Total Users	All-Person Consumption		All-Users Consumption	
				Mean (g)	90 th Percentile (g)	Mean (g)	90 th Percentile (g)
Infant	0-2	73.3	2,625	1.29	2.90	1.62	3.07
Children	3-11	99.8	6,292	2.68	4.48	2.68	4.48
Female Teenager	12-19	99.3	697	2.51	4.52	2.54	4.53
Male Teenager	12-19	99.4	692	3.42	6.22	3.45	6.28
Female Adult	20 and Up	98.4	4,501	1.90	3.47	1.93	3.50
Male Adult	20 and Up	98.6	4,684	2.63	4.90	2.66	4.92
Total Population	All Ages	94.6	19,491	2.34	4.37	2.39	4.40

Population Group	Age Group (Years)	% Users	Actual # of Total Users	All-Person Consumption		All-Users Consumption	
				Mean (mg/kg)	90 th Percentile (mg/kg)	Mean (mg/kg)	90 th Percentile (mg/kg)
Infant	0-2	73.1	2,499	102.6	222.6	129.0	237.3
Children	3-11	99.8	5,887	110.8	193.3	111.0	193.8
Female Teenager	12-19	99.3	681	46.2	87.6	46.6	87.7
Male Teenager	12-19	99.4	683	55.4	107.4	55.9	107.4
Female Adult	20 and Up	98.4	4,367	29.2	54.5	29.8	54.8
Male Adult	20 and Up	98.6	4,659	32.3	61.4	32.7	61.7
Total Population	All Ages	94.6	18,776	46.9	104.5	47.8	105.6

DATA SUPPORTING GRAS STATUS

The basis for the GRAS status of KiwiBerry Concentrate is scientific procedures based on the essential equivalence of the ingredient to the fruit from which it is derived, *A. arguta*, which has enjoyed years of safe consumption by humans, particularly in Asia. The safety of KiwiBerry Concentrate is supported by the relationship of hardy kiwi fruit to the common green kiwi, which also has been consumed historically. Taxonomical evidence indicates that hardy kiwi and green kiwi are closely related and an analytical comparison of KiwiBerry Concentrate and hardy kiwi fruit to green kiwi fruit demonstrates that they are all very similar in composition. The assessment of the safety of KiwiBerry Concentrate is further supported by the history of use of *A. arguta*, as the fresh fruit and/or cooked for jellies, sauces, pie fillings, etc., in Asia and also in the United States prior to 1958. Additionally, KiwiBerry Concentrate also is similar in composition to the golden kiwi (*Actinidia chinensis*), which is now commonly marketed as a whole fruit.

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Several unpublished, subchronic preclinical toxicity studies of KiwiBerry Concentrate (also referred to as PG102) were conducted, and the mutagenic potential of KiwiBerry Concentrate was investigated in the Ames test. Additionally, a single clinical trial using KiwiBerry Concentrate was conducted in adults with moderately severe atopic dermatitis. A limited number of published preclinical studies investigating other kiwi species were identified, and several other clinical studies that investigated the potential effect of kiwi on parameters such as laxative potential, platelet aggregation, plasma lipids, and antioxidants were identified and reviewed. Although some of these studies were not conducted specifically to assess the safety of kiwi, they demonstrated that kiwi was well tolerated and without side effects in humans. Collectively, these studies corroborate the GRAS status of KiwiBerry Concentrate.

COMPARATIVE ANALYSIS OF KIWIBERRY CONCENTRATE TO KIWIFRUIT

As mentioned, the major components of KiwiBerry Concentrate occur in the hardy kiwi fruit, all of which are present naturally in the human diet *via* consumption of the more common green kiwi fruit, and other fruits and fruit products. These major components include 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 vitamin C, several minerals (calcium, magnesium, phosphorus, potassium, and sodium), organic acids, and phenolic compounds. Processing of *A. arguta* to produce KiwiBerry Concentrate does not disproportionately concentrate any particular component, although fiber levels are greater in fresh fruit than in KiwiBerry Concentrate, most likely due to the fact that most fiber components are not water soluble, and hence the filtration step will remove the insoluble fiber.

Fructose, glucose, inositol, and sucrose are the major sugars present in KiwiBerry Concentrate. Very early in fruit development, hardy kiwi 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; Bolding *et al.*, 2000), and as the kiwi 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, followed by decreasing levels of fructose, glucose, and inositol, respectively (Klages *et al.*, 1998).

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 heat labile. The levels of organic acids present in KiwiBerry Concentrate are similar to those present in the fresh fruit.

Hardy kiwi, *A. arguta*, and the common green kiwi, *A. deliciosa*, descend the same taxonomical route, with each belonging to the genus, *Actinidia* Lindl. Although the hardy kiwi and green kiwi

are morphologically different, compositional analysis of 7 lots of KiwiBerry Liquid Concentrate revealed that the composition of KiwiBerry Concentrate remains proportional to that of the fresh hardy kiwi fruit, which is very similar to that of the common green kiwi when compared to published values identified in the scientific literature (see Attachment 2). Additionally, one lot each of *A. arguta* and *A. deliciosa* fruit were analyzed for composition. Proximate analysis was conducted, and levels of carbohydrates, organic acids, phenolic compounds, and minerals were measured. Summaries of the published and analytical values for the various components of KiwiBerry Concentrate, hardy kiwi, and green kiwi are presented in Tables 5 through 7.

Parameter	KiwiBerry Concentrate		<i>Actinidia arguta</i>		<i>Actinidia deliciosa</i>	
	Specification		Published Value ^b	Analytical Value ^c	Analytical Value ^d	
	Liquid	Powder				Liquid ^a
Moisture (%)	<50%	<6%	33.28 ± 6.1	NA	76.82	83.3
Ash (%)	<8%	<4%	4.64 ± 0.5	4.44	3.28	5.27
Protein (%)	<10%	<5%	5.91 ± 0.58	6.85 ± 0.13	6.17	6.65
Fat (%)	<10%	<5%	4.32 ± 2.14	6.62 to 6.69	7.38	11.08
Carbohydrate (%)	>70%	>75%	85.75 ± 2.83	NR	83.18	77.13
Total organic acids (mg/g)	>50	>25	NR	40	NR	NR
Calories (per 100 g)	NA	NA	399.7 ± 12.98	NR	422.78	437.13

* All measures are expressed on a dry weight basis except for moisture.

NA = Not applicable; NR = not reported

^a Mean ± standard deviation of 7 independent lots of manufactured concentrate. Raw material was Oregon-sourced fruit from 2 years harvest.

^b From Zhang *et al.* (1992). Values are the average of 3 measurements

^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 Range of pure (6.62%) to crude (6.69%) fat.

Carbohydrate Component	KiwiBerry Liquid Concentrate ^a (Mean ± SD)	<i>Actinidia arguta</i>		<i>Actinidia deliciosa</i>	
		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
Fiber	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	52.8
Glucose	23.06 ± 1.62	5.0 to 8.5	11.09	4.7 to 12.5	0.52
Inositol	4.99 ± 0.42	1.4 to 2.5	2.85	0.8	13.17
Sucrose ^e	0.30 ± 0.35	2.5 to 27.5	0.00	2.7 to 2.8	

Table 6 Carbohydrate, Organic Acid, and Mineral Components of KiwiBerry Liquid Concentrate in Comparison to Published and Analytical Values for <i>Actinidia arguta</i> and <i>Actinidia deliciosa</i> Fruit*					
Carbohydrate Component	KiwiBerry Liquid Concentrate ^a (Mean ± SD)	<i>Actinidia arguta</i>		<i>Actinidia deliciosa</i>	
		Published Values ^b	Analytical Value ^c	Published Values ^b	Analytical Value ^d
Organic Acids					
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 (ppm)	12.53 ± 20.83	DNR	149.70	DNR	452.50
Minerals^f					
Calcium (ppm)	1,267.9 ± 365	2,160	3,623	1,210	2,455
Magnesium (ppm)	1,131.9 ± 241	930	906	800	1,018
Phosphorus (ppm)	1,993 ± 527	NI	2,066	NI	3,180
Potassium (ppm)	21,204 ± 1,062	13,840	11,260	12,710	22,215
Sodium (ppm)	338 ± 140	NI	25.02	NI	77.84

* All measures are expressed on a dry weight basis; DNR = Did not research this topic; NI = Not identified

^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 on a wet basis (Zhang *et al.*, 1992; Boyes *et al.*, 1997a; 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.

^f Okamoto and Goto (2005).

Table 7 Phenolic Components of KiwiBerry Liquid Concentrate in Comparison to <i>Actinidia arguta</i> and <i>Actinidia deliciosa</i> Fruit*				
Phenolic Component	KiwiBerry Liquid Concentrate ^a		<i>Actinidia arguta</i> ^b	<i>Actinidia deliciosa</i> ^c
	Mean ± SD	Range		
Flavonoids (ppm)				
Quercetin	62.38 ± 22.57	21.5 to 82.4	36.07	16.71
Isorhamnetin	25.23 ± 3.22	ND to 31.4	ND	15.87
Kaempferol	23.74 ± 3.07	ND to 27.7	ND	12.1
Anthocyanins (ppm)				
Cyanidin	1.13 ^d	ND to 1.13	130.07	ND
Delphinidin	0.20 ± 0.07	ND to 0.32	9.15	0.18
Malvidin	5.9 ± 5.89	ND to 15.06	11.22	11.68
Pelargonidin	ND	ND	0.69	ND
Peonidin	0.18 ± 0.14	ND to 0.35	0.99	ND
Catechins	ND	ND	ND	Not tested

* All measures are expressed on a dry weight basis; ND = None detected

^a Mean ± standard deviation of 7 independent lots of manufactured concentrate with the exception of catechins (1 lot tested). 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.

^d Cyanidin detected in only 1 of 7 lots tested.

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HISTORY OF USE OF *ACTINIDIA ARGUTA*

Hardy kiwi fruit is similar in taste to the common green kiwi fruit, but is smaller and with fuzzless, smooth skin (Strik and Cahn, 1996). The genus *Actinidia* is native to eastern Asia, where the distribution of *A. arguta* extends from Japan through northeastern Asia (Korea, eastern Siberia, and Manchuria) and through much of China, and the fruit is cultivated in these countries with a documented history of human consumption (Dunn, 1911; Darrow and Yerkes, 1937; Michurin, 1949; Li, 1952; Titlyanov, 1963; Ferguson, 1990, 1991; Zhang *et al.*, 1992; Anetai *et al.*, 1996; California Rare Fruit Growers, Inc., 1996; Boyes *et al.*, 1997b; Kolbasina, 2000; Mansfeld, 2001).

Documentation of use of the fresh fruit is very extensive; however, despite the documented historical consumption of hardy kiwi, quantitative consumption data have not been identified. 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 (Dunn, 1911; Nakai, 1933; Li, 1952; Anetai *et al.*, 1996). The KiwiBerry Concentrate ingredient is most comparable to a jam or jelly-type product since these contain cooked fruit concentrates. Use of the dried and cooked fruit is documented from sources in the United States (Darrow and Yerkes, 1937) and from Europeans living in Siberia (Titlyanov, 1963), and the use of *A. arguta* in jams and jellies would likely have yielded a cooked product¹ with a water content of 30 to 40%, which is comparable to the water content of the KiwiBerry Liquid Concentrate. There also is documentation of use of a dried powdered product as a traditional Chinese medicine (Zhang *et al.*, 1992).

Evidence of *Actinidia* spp. growth and consumption in China and Japan was reported by Dunn (1911), who stated that 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." 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). Regarding the food use of *A. arguta* fruit in China, Li (1952) reported that "*A. arguta*, well known as Yang-tao in China, (has) long been used for (its) edible fruits, which have a greenish pulp of pleasant acid taste." Zhang *et al.* (1992) reported that the fruit extracts, prepared by boiling dried fruits, are used traditionally in China to improve digestion and general health. In Korea, hardy kiwi fruit is described as being consumed as a food and used as an herbal medicine, 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).

Cultivation of *A. arguta* in Siberia dates back to 1930 when the Russian scientist, Mičurin, was reported to make selections of *A. arguta* for food use over a period of several decades (Titlyanov, 1963; Mansfeld, 2001), with reports of use of the fruit being eaten fresh or in dried or cooked form, including as jams, and also as an ingredient to make wine (Titlyanov, 1963).

¹ Water content of multiple jams and jellies adapted from Souci *et al.* (1989).

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.

The cultivation of *A. arguta* in the United States, as well as *A. chinensis*, 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 the southeastern U.S. as well as California. The kiwi fruits were consumed fresh and/or used for jellies and sauces. Numerous agricultural scientists acknowledge that *A. arguta* is currently cultivated in the U.S. and produces edible fruits, and various publications pertaining to the cultivation methods of hardy kiwi fruit are available (Strang and Funt, 1993; Strik and Cahn, 1996; Penn State, 2001), and the United States Department of Agriculture (USDA) Agricultural Research Service (ARS) lists *A. arguta* as a minor crop².

Hardy kiwi is now also cultivated in Canada, China, France, Germany, and Italy (Zhang *et al.*, 1992; Strik and Cahn, 1996; Ferguson, 1999; Strik, 2002), and researchers in New Zealand reported that *A. arguta* is widely grown across the Northern Hemisphere due to its hardiness, and is highly desirable due to its sweet taste (Boyes *et al.*, 1997b). *A. arguta* is used in the production of sauces, wine, jam, and desserts.

Due to the similarity in composition between the hardy kiwi and other species of kiwi, it is useful to compare the background consumption of kiwi to intakes of KiwiBerry Concentrate. The *per capita* kiwi consumption in the U.S. was reported to be as high as 0.59 lbs/year in 1993 (USDA, 2004), or approximately 3.56 g/person/day. On a dry weight basis, the composition of KiwiBerry Concentrate to the fresh kiwi fruit is proportional, and hence, the estimated all-person exposure to KiwiBerry Concentrate (2.34 g/day) is within range of the reported *per capita* consumption of kiwi in the U.S.

METABOLIC FATE

As previously stated, the KiwiBerry Concentrate comprises mainly carbohydrate (at least 70%), consisting primarily of sugar (*i.e.*, fructose, glucose, inositol, and sucrose), with lesser amounts of protein and fat (10% of each). These major macronutrients present in KiwiBerry Concentrate are expected to undergo normal metabolism. The minor components in KiwiBerry Concentrate, such as organic acids and phenolics, also are expected to undergo normal metabolism and therefore none of the constituents pose a risk to safety.

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

STUDIES CORROBORATING THE SAFETY OF KIWIBERRY CONCENTRATE

Studies with KiwiBerry Concentrate

Toxicological Studies

KiwiBerry Concentrate was negative for mutagenicity in the Ames assay using *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535, and TA1537 in the presence or absence of metabolic activation (S9), at all concentrations up to 5,000 µg/plate (MDS Pharma Services, 2004).

Various subchronic studies of KiwiBerry Concentrate were conducted by PanGenomics & SNU (Korea), which 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). An additional 76-day subchronic gavage toxicity study in rats was conducted by WIL Research Laboratories (Ashland, OH), in accordance with good laboratory practice (GLP) (WIL Research Laboratories, 2005). A summary of these studies is presented in Table A3-1 (see Attachment 3).

KiwiBerry Concentrate administered to 10 female balb/c mice daily by gavage at dose levels of 0 or 150 mg/kg body weight for a period of 28 days was reported to have no effects on mortality, clinical signs, body weight gains, or hematological and clinical chemistry analyses (PanGenomics Co., Ltd. and Seoul National University, 2002). Additionally, no abnormalities were reported following histological analysis of the kidney, spleen, thymus, and liver of all animals. The No Observed Effect Level (NOEL) and the No Observed Adverse Effect Level (NOAEL) is 150 mg/kg body weight/day, the only dose tested.

KiwiBerry Concentrate administered orally (presumably by gavage but not specified in the report) to groups of 5-week-old balb/c mice (7/sex/group) at dose levels of 0 or 150 mg/kg body weight/day for a period of 3 months was reported to produce no compound-related effects on mortality or clinical signs (PanGenomics Co., Ltd. and Seoul National University, 2003a). 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 hematological parameters were assessed, and some differences were reported between groups. The serum concentrations of total protein, albumin, total bilirubin, total cholesterol, phosphorus, and chloride were reportedly significantly decreased in treatment males compared to controls, while concentrations of phosphorus and sodium were significantly decreased in females. Creatinine and blood urea nitrogen (BUN) also were reported to be significantly increased in females compared to controls. Additionally, organ weight analyses revealed lower kidney, liver, and heart weights in treated 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. It may be concluded that the NOAEL is 150 mg/kg body weight/day, the only dose tested.

No significant effects on mortality, clinical signs, or body weight gains were reported in 5-week-old Sprague-Dawley rats (7/sex/group) administered KiwiBerry Concentrate orally (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). Additionally, there were no histopathological findings in either the liver or heart (the only organs examined). Transient but statistically significant changes in clinical chemistry were reported at 100 and 300 mg/kg body weight/day in both sexes; however, the authors considered these changes not toxicologically significant as the values remained within normal reference ranges. There were no abnormalities identified following histological examination of all KiwiBerry Concentrate-treated rats (PanGenomics Co., Ltd. and Seoul National University, 2004). Following hematological examination there were no significant changes in males dosed with KiwiBerry Concentrate. A few changes were reported in the hematological parameters of female rats; however, these were transient and non-dose-dependent and were considered not toxicologically significant by the authors due to a lack of supporting histological evidence. A number of sporadic changes in both absolute and relative organ weights were reported, but these were not clinically significant according to the authors. Slight focal cellular infiltration of the liver was observed in one high-dose male and in 1, 4, and 2 females from the low-, mid-, and high-dose groups, respectively, and in 1 male and 2 female control rats. Similarly, slight focal cellular infiltration of the heart was reported in a single male of each of the KiwiBerry Concentrate treated groups. Focal myocardial degeneration also was reported in a single high-dose treated male rat. This lesion occurs spontaneously in Sprague-Dawley rats and is not indicative of toxicity (Greaves and Faccini, 1992). The authors stated that no abnormal findings were observed 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. It may be concluded that the NOAEL is 1,000 mg/kg body weight/day, the highest dose tested.

WIL Research Laboratories (2005) conducted a 76-day (approximately 2.7 months) GLP subchronic study in CrI:CD rats with KiwiBerry Liquid Concentrate. Rats (20/sex/group) were administered KiwiBerry Concentrate in 1% hydroxypropyl methylcellulose (vehicle) by gavage at doses of 0, 500, 1,000, or 2,000 mg KiwiBerry Concentrate/kg body weight/day during postnatal Days (PNDs) 8 to 84. A separate group of control rats (20/sex) was provided the vehicle alone under the same treatment conditions. Fifteen (15) rats/sex/group underwent necropsy one day following the end of the treatment period (*i.e.*, PND 85), while the remaining 5 rats/sex/group were necropsied following a 4-week non-dosing period (*i.e.*, PND 113). Developmental

parameters (balanopreputial separation and vaginal patency) were assessed beginning during the second week of the study, females beginning at PND 25 and males beginning at PND 35. Functional observation battery (FOB) assessments (e.g., behavioral, sensory, and neuromuscular observations) also were conducted during Week 10 of the study period, and full hematological and biochemical evaluations were conducted just prior to necropsy in all rats. Additionally, absolute organ weights and organ weights relative to brain or final body weight 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.

No compound-related deaths were reported in any of the rats 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. The administration of KiwiBerry Concentrate had no effect on food consumption. Although transient changes in body weight were reported, including a significant decrease in mean body weight gain in females of the 500 mg/kg body weight/day group between PNDs 39 to 42, 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 PNDs 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. The authors concluded 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 PND 113. This effect was not observed in females at this dose at PND 113, nor in males or females dosed with 2,000 mg/kg body weight/day and necropsied on PND 85. Mean cholesterol was significantly reduced in males in the 500 mg/kg body weight/day dose group compared to control rats at PND 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 PND 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 hematological or biochemical parameters tested.

At PND 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 PND 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 that these effects on organ weights were not toxicologically significant. Females dosed with 1,000 or 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 PND 113. Similar effects were not reported in any rat at PND 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 PND 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 tumor 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.

Groups of 5-week-old Sprague-Dawley rats (7/sex/group) administered KiwiBerry Concentrate orally (presumably by gavage but route not specified) at dose levels of 0 or 300 mg/kg body weight/day for a period of 6 months were reported to exhibit no significant effects on mortality, clinical signs, or body weight gains, although one female treated rat died from side effects of anesthesia (PanGenomics Co., Ltd. and Seoul National University, 2003b). Biochemical analyses revealed decreased glucose and increased sodium levels in treated males compared to control males. In KiwiBerry Concentrate-treated females, the activity of glutamic pyruvic transaminase (GPT) and amylase were increased compared to control females, while the concentration of calcium was decreased. The numbers of eosinophils were reportedly increased in males at the time of testing of hematological parameters. Organ weight analyses revealed decreased relative testis 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; therefore, the NOAEL is 300 mg/kg body weight/day, the only dose tested.

Clinical Study

A single randomized, double blind, placebo-controlled study was conducted in adults to investigate the effect of KiwiBerry Concentrate on the signs and symptoms of moderately severe atopic dermatitis (Mraz *et al.*, 2005). Each subject [n=51; 19 men and 32 women, 19 to 65 years of age; mean age 45 (treatment group), 34 (placebo group)] was provided an oral dose of either KiwiBerry Concentrate (600 mg/day) or placebo (microcrystalline cellulose) for a period of 42 days. A total of 46 subjects completed the study, and 43 subjects (16 men and 27 women) were included in the statistical analysis. No significant changes were reported following assessments of full blood chemistry, hematology, or urinalysis, which were conducted on Days 1, 14, and 42. Furthermore, no serious adverse events were reported by either group, and the incidence and severity of events reported was 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. KiwiBerry Concentrate was therefore reported by the authors to be well tolerated. A summary of this study is presented in Table A3-1 (see Attachment 3).

Other Identified Studies of Kiwi

Preclinical 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, with an additional group of rats serving as the control group. At 5 weeks, blood glucose was reported to be significantly decreased compared to baseline in all rats treated with kiwi; 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 (Han *et al.*, 2004).

Panjehshahin *et al.* (2003) investigated the effect of a hydro-alcoholic extract of kiwi (species not specified) on the structure of reproductive tissues in male rats (10/group; strain not

specified). 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 testes had become fusiform. 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 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 and the full findings of this study do not appear to have been published. The toxicological significance of these findings is unclear.

Panjeh-Shahin *et al.* (2005) investigated the effect of a hydro-alcoholic extract of *A. chinensis* on sperm count and motility and serum levels of estradiol and testosterone. Male Sprague-Dawley 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 findings are not clear. Furthermore, the findings of these studies are not corroborated by the history of safe consumption of kiwi 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).

Traditional mutagenicity/genotoxicity or carcinogenicity studies on kiwi fruit or extract were not identified in the literature; however, the antimutagenic potential of kiwi fruit juice and extract has been reported in *S. typhimurium* (Edenharder *et al.*, 1994; Ikken *et al.*, 1999), as were antigenotoxic effects of kiwi fruit juice in Chinese hamster lung fibroblasts (Edenharder *et al.*, 2002). Additionally, kiwi fruit was reported to exert strong anticlastogenic effects against benzo[a]pyrene in the *in vivo* mouse bone marrow micronucleus assay (Edenharder *et al.*, 1998). Extracts of kiwi fruit showed cytotoxic activity against human oral tumor lines (Motohashi *et al.*, 2002), and kiwi fruit juice was reported to provide protection against oxidative DNA damage in human lymphocytes *ex vivo* and *in vitro* (Collins *et al.*, 2001).

Clinical Studies

A single clinical study was identified that investigated several safety parameters following consumption of kiwi juice by elite Chinese athletes (ages 18 to 32, gender not reported) (Di *et al.*, 1990). Athletes were provided a kiwi fruit drink supplement or placebo (ranging from 500 to 1,200 mL)³, during 2 separate training sessions, where half of the total volume was provided 10 minutes prior to training, and the rest was provided halfway through the training session. 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 electrocardiogram, and 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.

Additional clinical studies designed to investigate effects of kiwi on laxation, platelet aggregation, plasma lipids, and antioxidants were conducted in healthy male and female adult volunteers including seniors using fresh kiwi fruit and kiwi juice. Both were well tolerated with no reported side effects. (Collins *et al.*, 2001, 2003; Rush *et al.*, 2002; Duttaroy and Jorgensen, 2004). The studies ranged in length from 1 day to 28 days and included daily doses of up to 3 whole kiwi fruit or 1 whole kiwi fruit (100 g)/30 kg body weight (average body weight of the subjects was approximately 69 kg).

KIWI ALLERGENICITY

Allergy to kiwi, primarily *A. deliciosa*, has been documented, with identified reported cases of localized oral allergy syndrome (OAS), most commonly characterized by itching and swelling of the lips, mouth, and throat, but also more severe anaphylaxis (CFIA, 2000; Lucas *et al.*, 2003). Some individuals with kiwi fruit allergy seem also to be sensitive to pollens (such as birch and grass pollen), apples, melon, and latex, which are cross-reactivity allergies that are commonly referred to as pollen-food allergy syndrome or latex-food allergy syndrome (Möller *et al.*, 1997; Pastorello *et al.*, 1998; Rodriguez *et al.*, 2000); however, 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 (Bublin *et al.*, 2004; Lucas *et al.*, 2004). *In vitro* examination of the potential for cross-reactivity between hardy kiwi (*A. arguta*) allergens and allergens present in green and golden kiwi using immunoglobulin E (IgE) immunoblots and the direct enzyme-linked immunosorbent assay (ELISA) revealed that at least some individuals with known allergy to green kiwi are likely to experience allergic reactions to fresh hardy kiwi fruit following consumption (Chen *et al.*, 2006); however, there is evidence to suggest that kiwi allergens are heat labile, and thus heated KiwiBerry Liquid Concentrate appears to lack allergenic potential,

³ The composition of the drink was: carbohydrate, 5%; potassium, 4 to 8 mEq/L; sodium, 25 mEq/L; magnesium, 2 mEq/L; calcium, 3 mEq/L; iron, 1 mg%; chloride, 38 mEq/L; vitamin C, 48 mg%.

although Chen *et al.* (2006) reported that "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". Since KiwiBerry Concentrate will be labeled as originating from hardy kiwi fruit, it is anticipated that individuals with kiwi allergy will avoid ingestion of this product.

SUMMARY

Efficas intends to market KiwiBerry Concentrate, a concentrate from hardy kiwi fruit, as a food ingredient in a variety of food products such as baked goods and mixes, breakfast cereals, chewing gum, dairy product analogs, grain products and pastas, candies, jams and jellies, milk and plant protein products, snack foods, beverages products, and processed fruits and vegetables and their juices. Hardy kiwi fruit, *A. arguta*, is similar in taste to the common green kiwi fruit, *A. deliciosa*, but is smaller and with fuzzless, smooth skin (Strik and Cahn, 1996). Hardy kiwi is indigenous to northern China, Japan, Korea and Siberia and is cultivated in China, Europe, Japan, Korea, New Zealand, Siberia, and the United States, and has a documented history of human consumption (Dunn, 1911; Darrow and Yerkes, 1937; 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). Despite the documented historical consumption of hardy kiwi, quantitative consumption data have not been identified.

KiwiBerry Concentrate is manufactured in accordance with cGMP and meets appropriate food-grade specifications. Essentially, the Concentrate is produced *via* hot water extraction of dried hardy kiwi, with subsequent purification of the crude concentrate to produce the end product. For every 100 g of fresh hardy kiwi, 4 to 5 g of KiwiBerry Liquid Concentrate (on a dry weight basis) is produced, depending on the water content of the fruit. 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 consistent product, Efficas has established numerous chemical and microbiological specifications for the final preparation, and batch samples are routinely assayed to verify that the set limits are met, ensuring a safe consistent product. Additionally, stability studies on KiwiBerry Concentrate itself and on the concentrate as a component of a food matrix (*i.e.*, granola bar) indicate it is very stable at room temperature and under accelerated conditions for up to 3 months.

Under the conditions of intended use, the total population all-user mean intake of KiwiBerry Concentrate was estimated to be 2.39 g/person/day or 47.8 mg/kg body weight/day. The heavy consumer (90th percentile) all-user intake of KiwiBerry Concentrate from all proposed food-uses was estimated to be 4.40 g/person/day or 105.6 mg/kg body weight/day. Background consumption of kiwi in the United States has been reported to be as high as 3.56 g/person/day (USDA, 2004), and on dry weight basis, the composition of KiwiBerry Concentrate is proportional to that of the fresh fruit, and the all-person estimated intake of the ingredient is

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within range of the calculated *per capita* consumption of kiwi in the U.S. KiwiBerry Concentrate comprises mainly carbohydrates (at least 70%), including sugars (fructose, glucose, inositol, and sucrose), starch, fiber, with a minor amount of protein and fat (less than 10% of each), as well as organic acids and phenolics, all of which have a long history of consumption as part of a normal diet.

The basis for the GRAS status of KiwiBerry Concentrate is scientific procedures based on the proportional composition of the concentrate to the fresh hardy kiwi fruit itself. This is evidenced by published analytical data of the fruit as well as a comparative analysis of KiwiBerry Concentrate and hardy kiwi fruit that demonstrates that on a dry weight basis, the concentrate is proportionally similar to fresh hardy kiwi fruit, which has a documented history of consumption. *A. arguta*, as the fresh fruit and/or cooked for jellies, sauces, pie fillings, etc., has a long, documented history of consumption in Asia and also in the United States prior to 1958. Hardy kiwi is indigenous to northern China, Japan, Korea and Siberia and is cultivated in China, Europe, Japan, Korea, New Zealand, Siberia, and the United States, and has a documented history of human consumption (Dunn, 1911; Darrow and Yerkes, 1937; 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).

Additionally, comparative analysis of KiwiBerry Concentrate and hardy kiwi to green kiwi fruit demonstrates they are very similar in composition and green kiwi fruit has a history of consumption in Asia as well as Australia, North America, and Europe. Furthermore, taxonomical evidence indicates that hardy kiwi and fuzzy kiwi are closely related. The assessment of the safety of KiwiBerry Concentrate is therefore supported by the relationship of hardy kiwi fruit to the common green kiwi, which has enjoyed years of safe consumption by humans, as well as. 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, subchronic preclinical toxicity studies were conducted using KiwiBerry Concentrate (also referred to as PG102), and collectively, these studies corroborate the safety of KiwiBerry Concentrate (see Table A3-1, Attachment 3). In one study, Crl:CD rats were provided gavage doses of up to 2,000 mg of KiwiBerry Concentrate/kg body weight/day for a period of 76 days without any toxicologically significant effects (WIL Research Laboratories, 2005). The authors reported a NOAEL of 2,000 mg/kg body weight/day for KiwiBerry Concentrate, the highest dose tested. Additionally, oral administration of 150 mg KiwiBerry Concentrate/kg/body weight/day for up to 3 months did not produce any toxicologically significant effects in mice, and similarly, administration to rats of 1,000 and 300 mg KiwiBerry Concentrate/kg body weight/day for periods of 28 days and 6 months, respectively, did not produce any compound-related adverse effects. 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).

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, hematology, or urinalysis, or in any adverse effects in adults with moderately severe atopic dermatitis, and 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) (see Table A3-2, Attachment 3). Kiwifruit 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 green kiwi has been documented, with symptoms ranging from rather mild cases of oral allergy syndrome to severe, systemic anaphylaxis following consumption. Additionally, some individuals with kiwi fruit allergy seem also to be sensitive to pollen, apples, melon, and latex, which are 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 that demonstrates that kiwi allergens are heat labile, and heated KiwiBerry Liquid Concentrate will likely lack allergenic potential; however, as an oral challenge was not conducted on kiwi-allergic patients in this trial, it is appropriate to be cautious in the interpretation strictly of *in vitro* IgE binding observations, and Chen *et al.* (2006) reported that "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". Since KiwiBerry Concentrate will be labeled 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, 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 published and unpublished data and information summarized in this report demonstrate that KiwiBerry Concentrate, meeting appropriate food-grade specifications and manufactured in accordance with cGMP, is GRAS for the conditions of intended use described herein based on scientific procedures. This statement is based on the essential equivalence of KiwiBerry Concentrate to the fresh hardy kiwi fruit, for which the historical consumption has been documented, and is supported by the compositional similarity between hardy and green kiwi, the composition of the ingredient itself, and pre-clinical and clinical trials conducted with KiwiBerry Concentrate.

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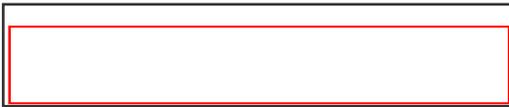
CONCLUSION

We, the Expert Panel, have independently and collectively critically evaluated the data and information summarized above and conclude that KiwiBerry Concentrate derived from hardy kiwi, *Actinidia arguta*, meeting appropriate food-grade specifications and manufactured in accordance with cGMP, is Generally Recognized as Safe (GRAS) under the conditions of intended use in foods specified herein, based on scientific procedures.



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Expert Panel CVs

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ATTACHMENT 1

CURRICULA VITAE OF EXPERT PANEL MEMBERS

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Joseph Francis Borzelleca



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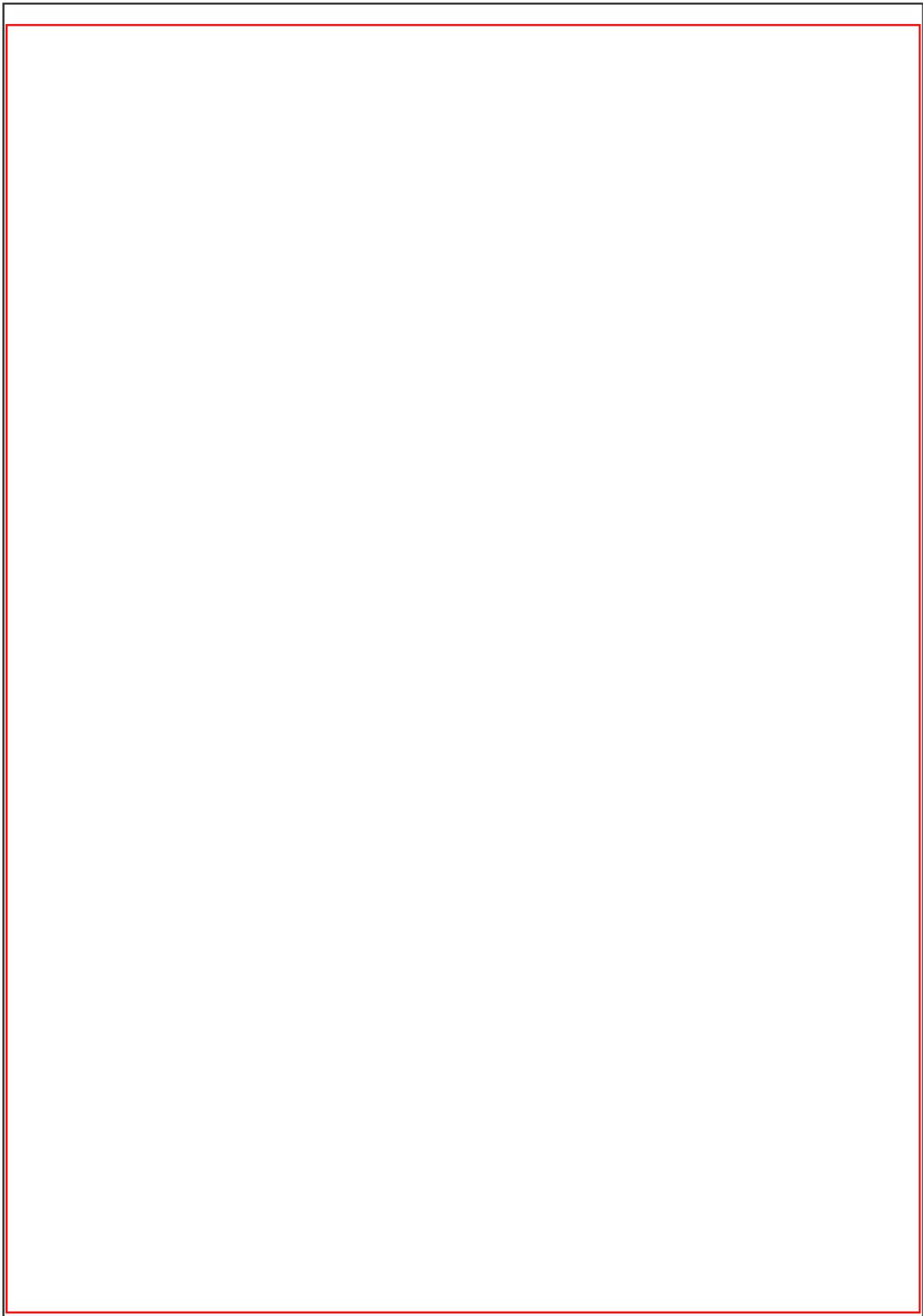


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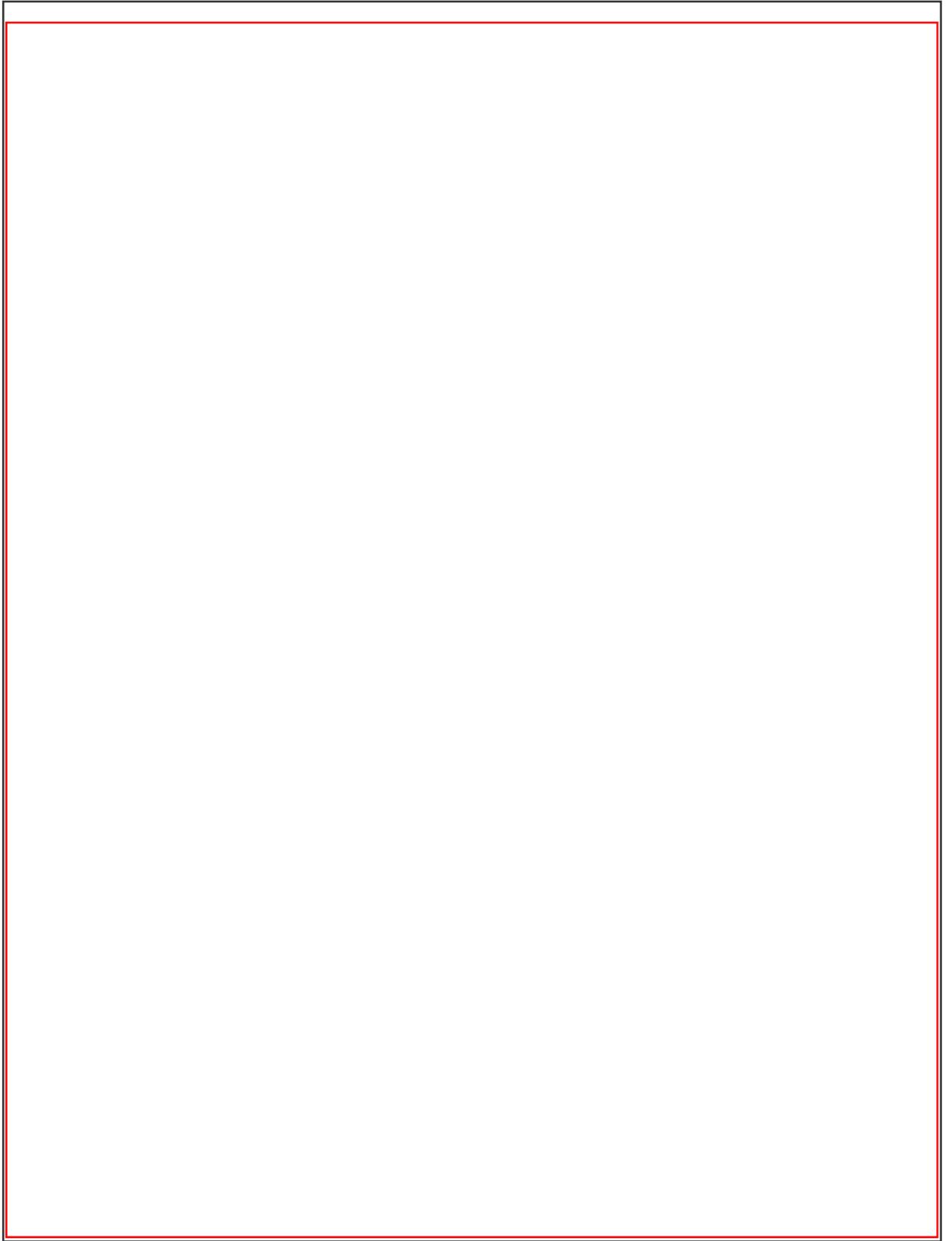


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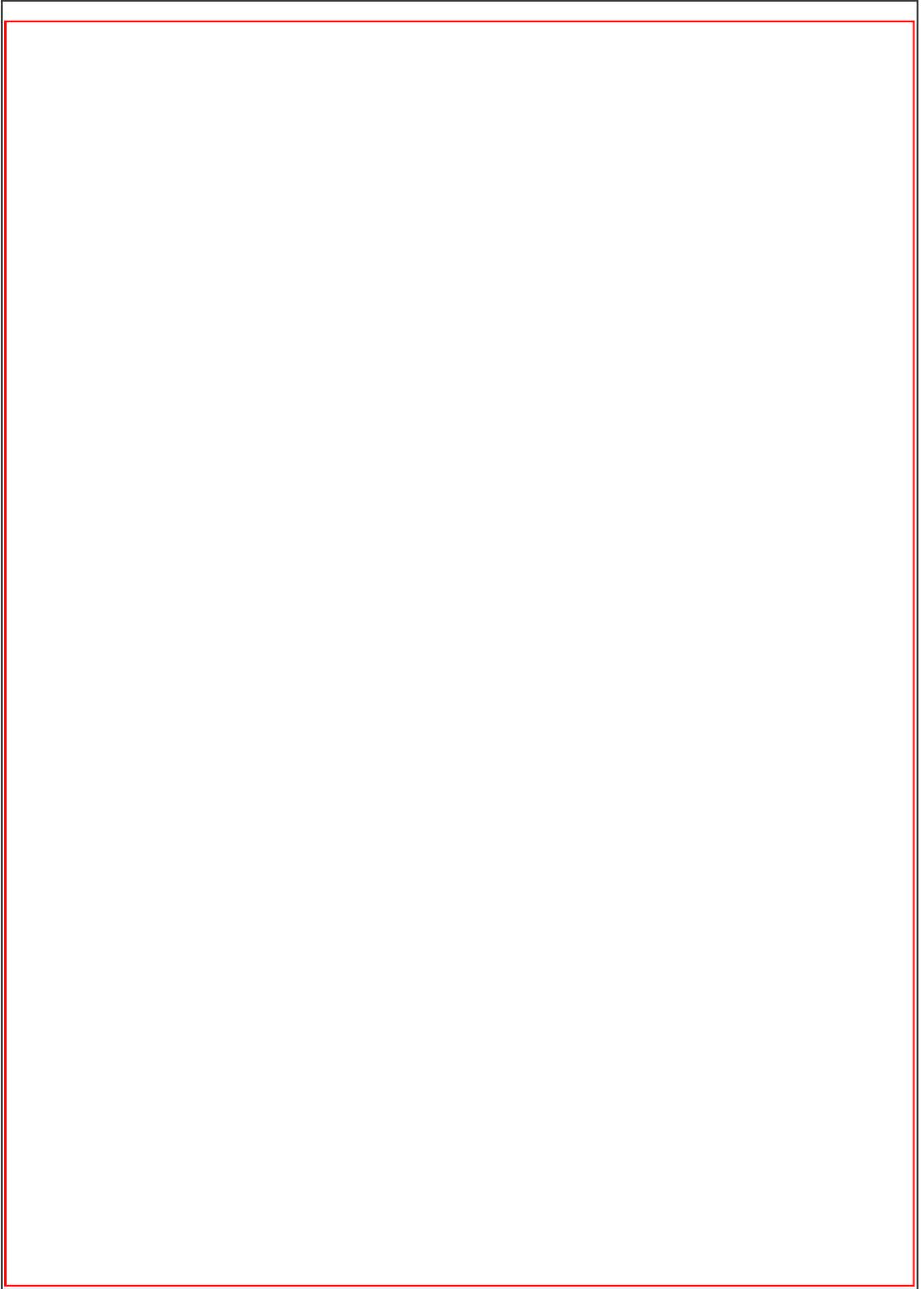


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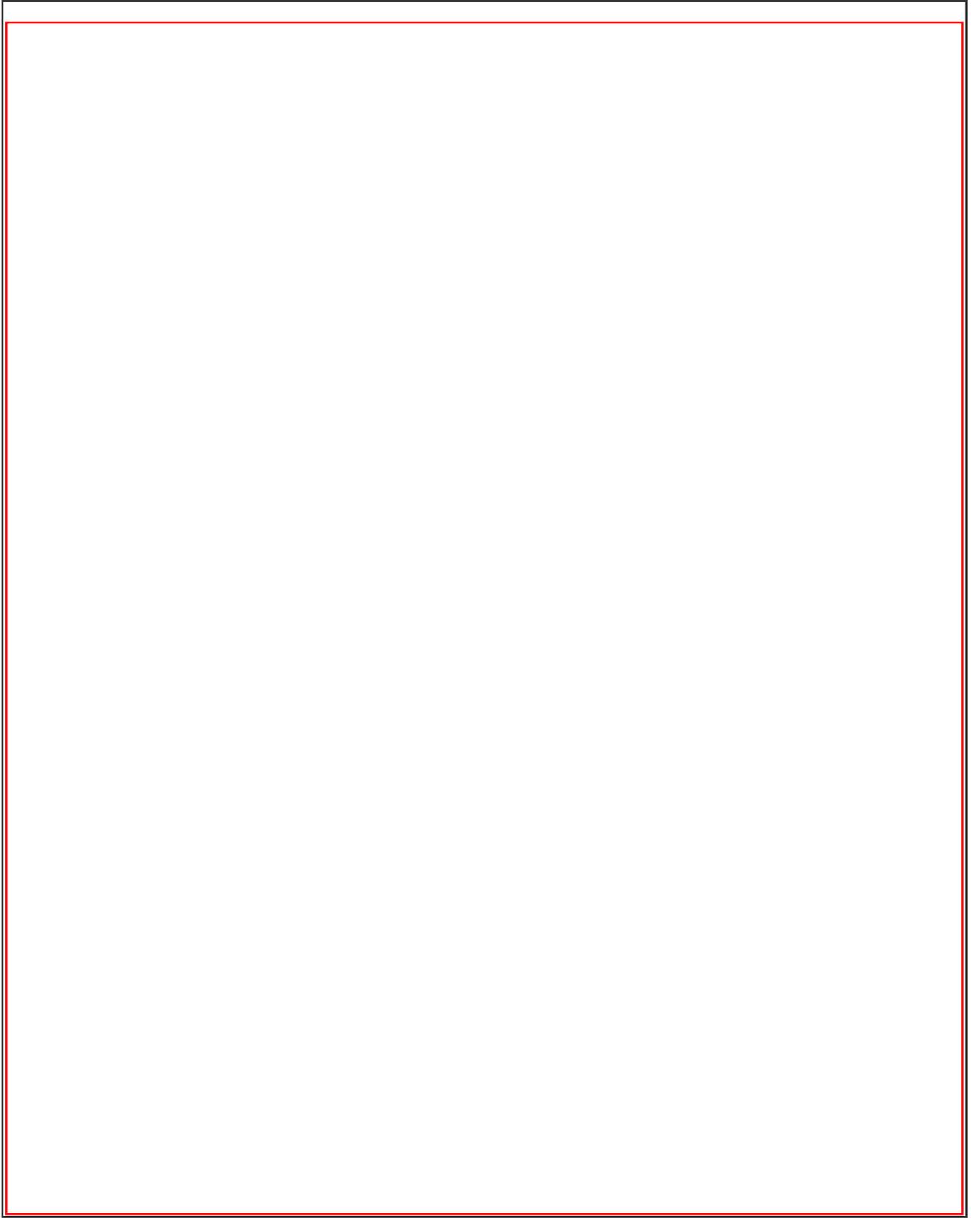
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CURRICULUM VITAE

NAME

Robert J. Nicolosi

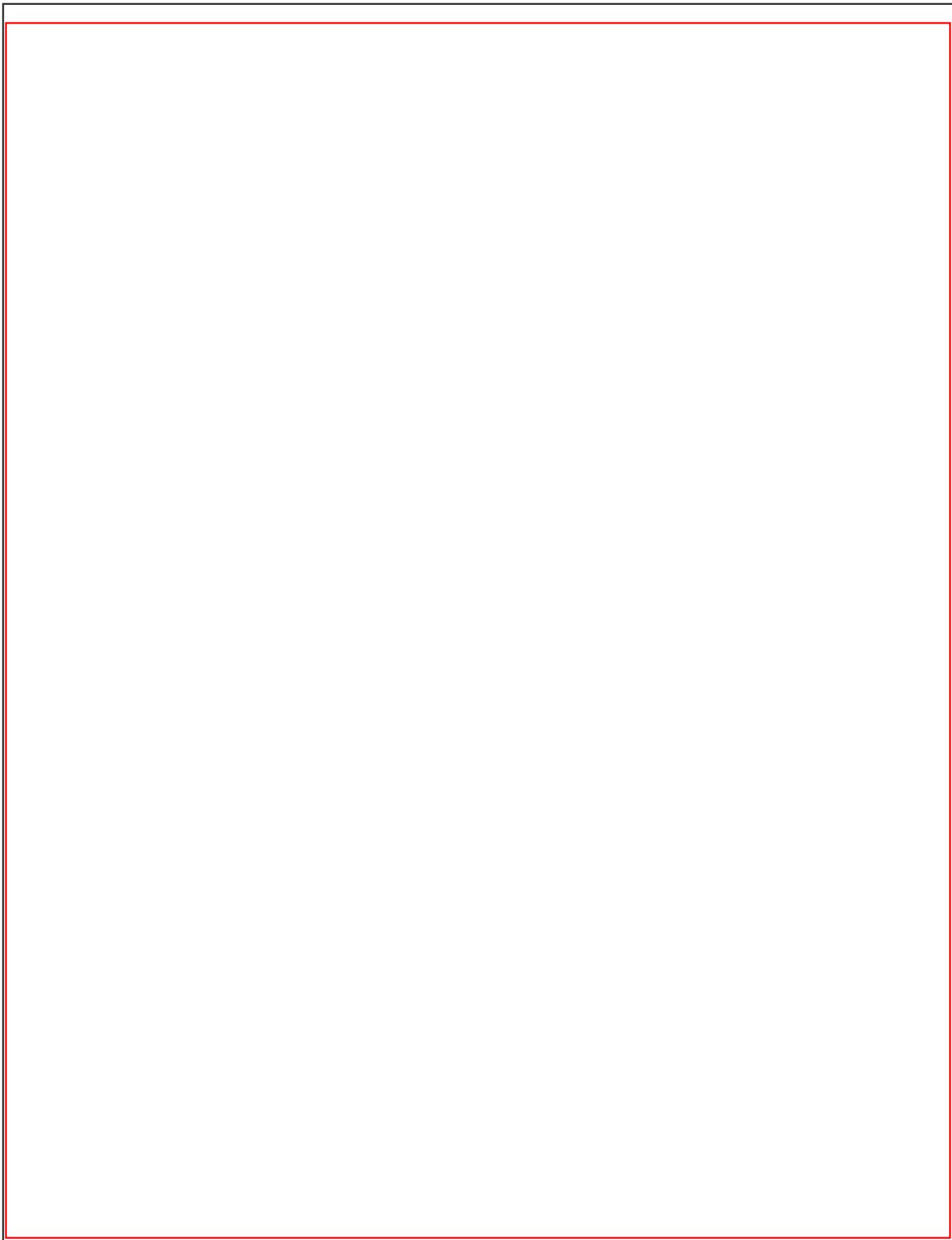
POSITION/TITLE

**Director - Center for Health & Disease
Research
Professor - Department of Health and
Clinical Sciences**

EDUCATION

YEAR

	YEAR



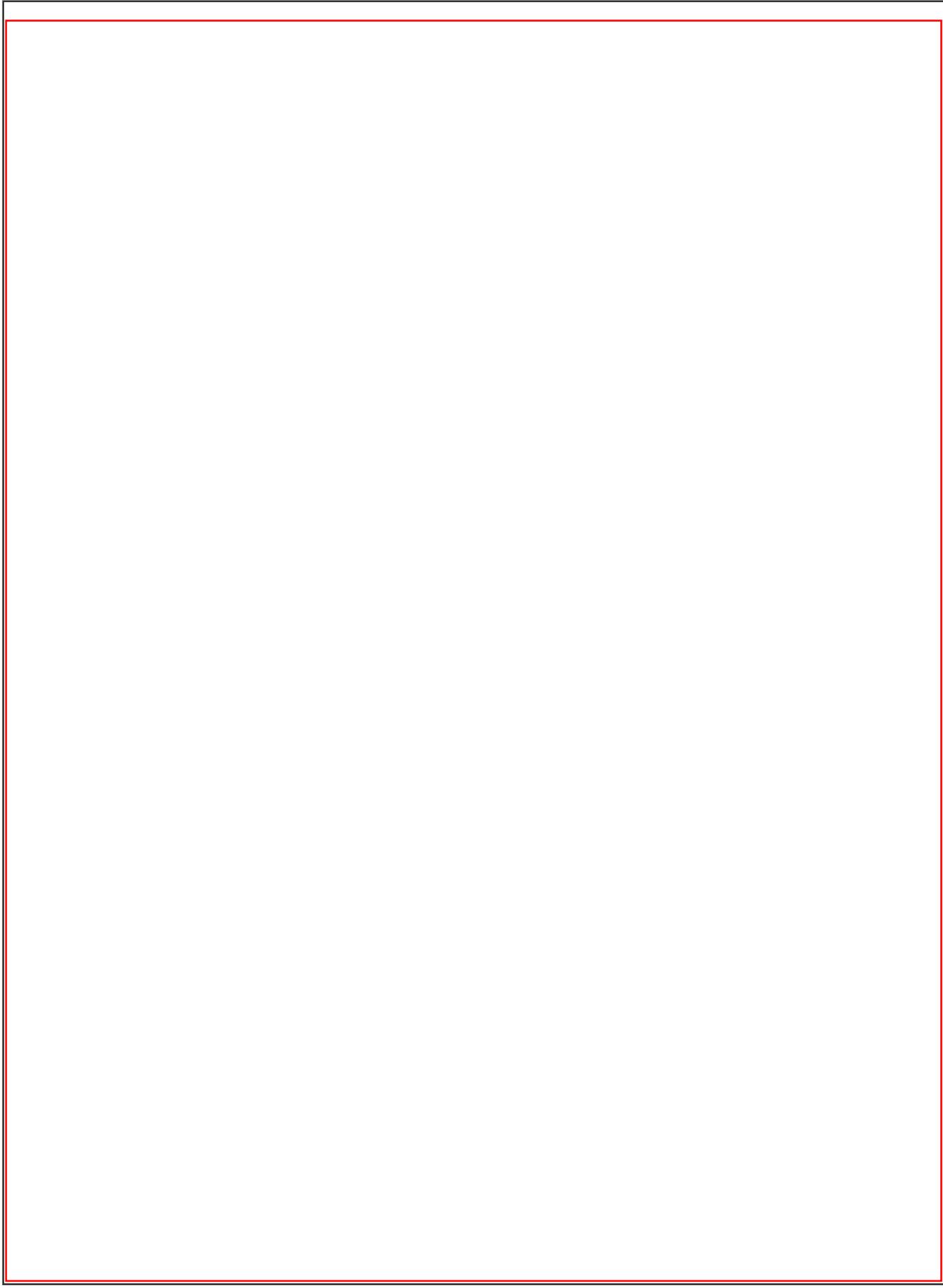




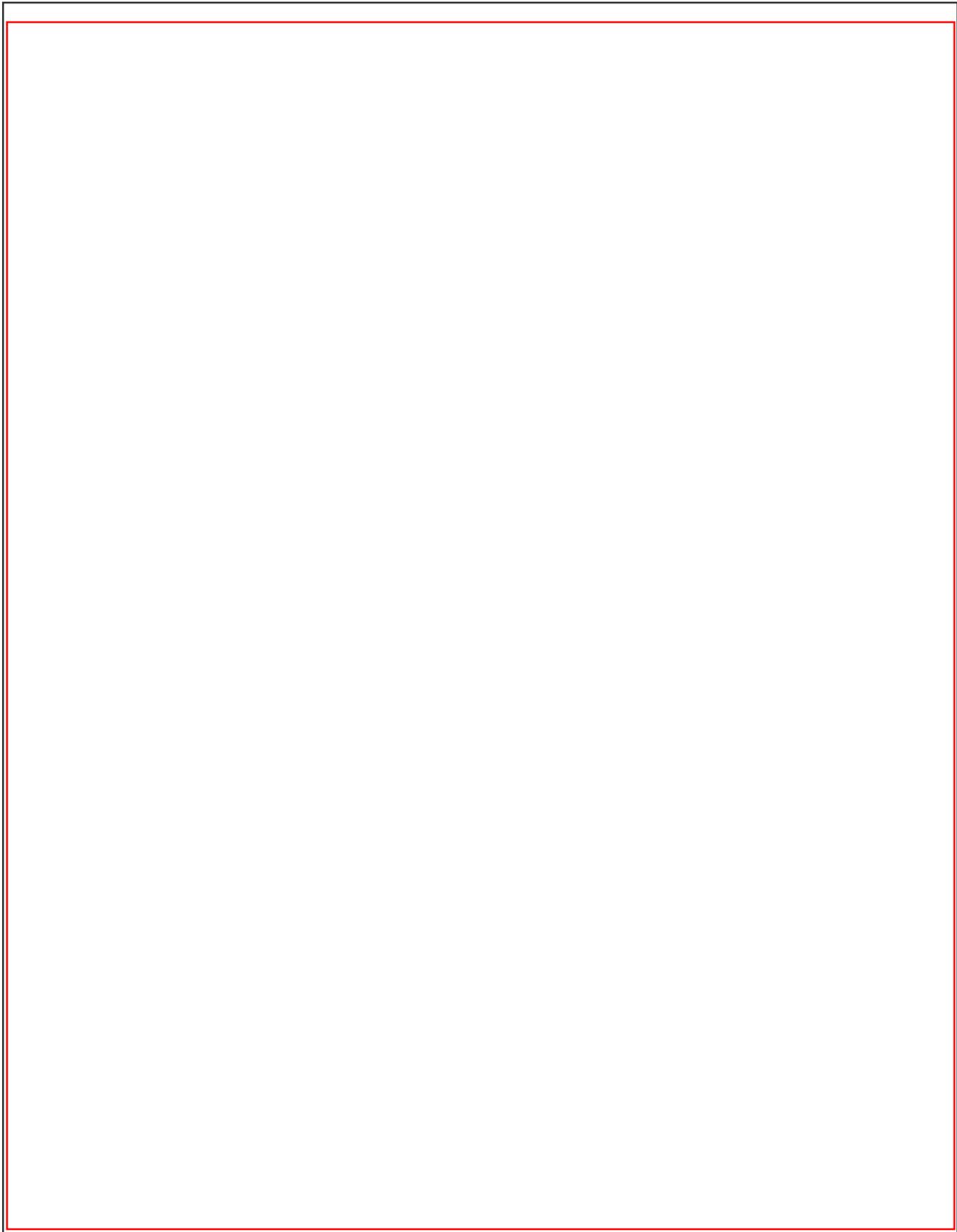














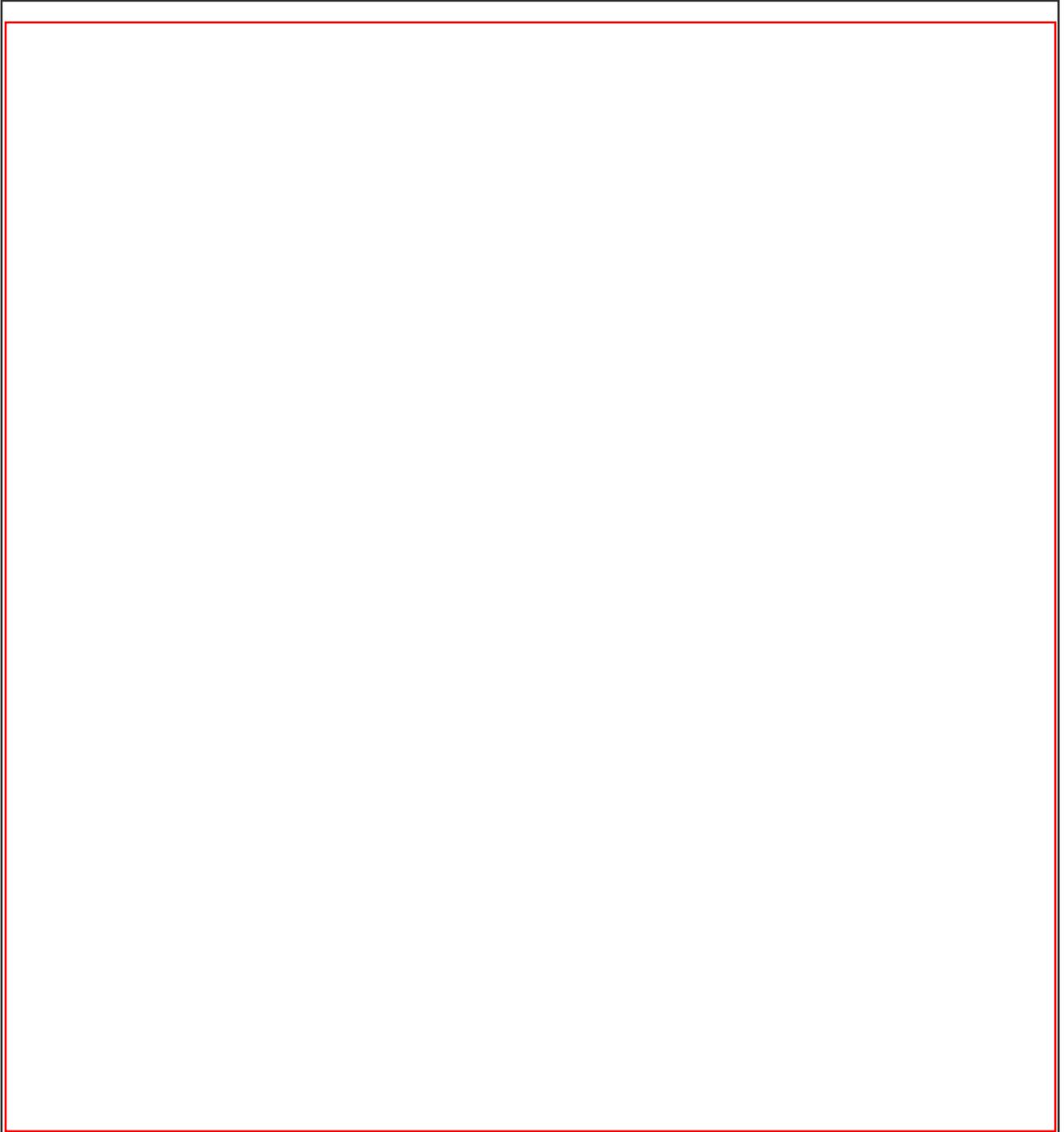
CURRICULUM VITAE

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Maxcy Distinguished Professor



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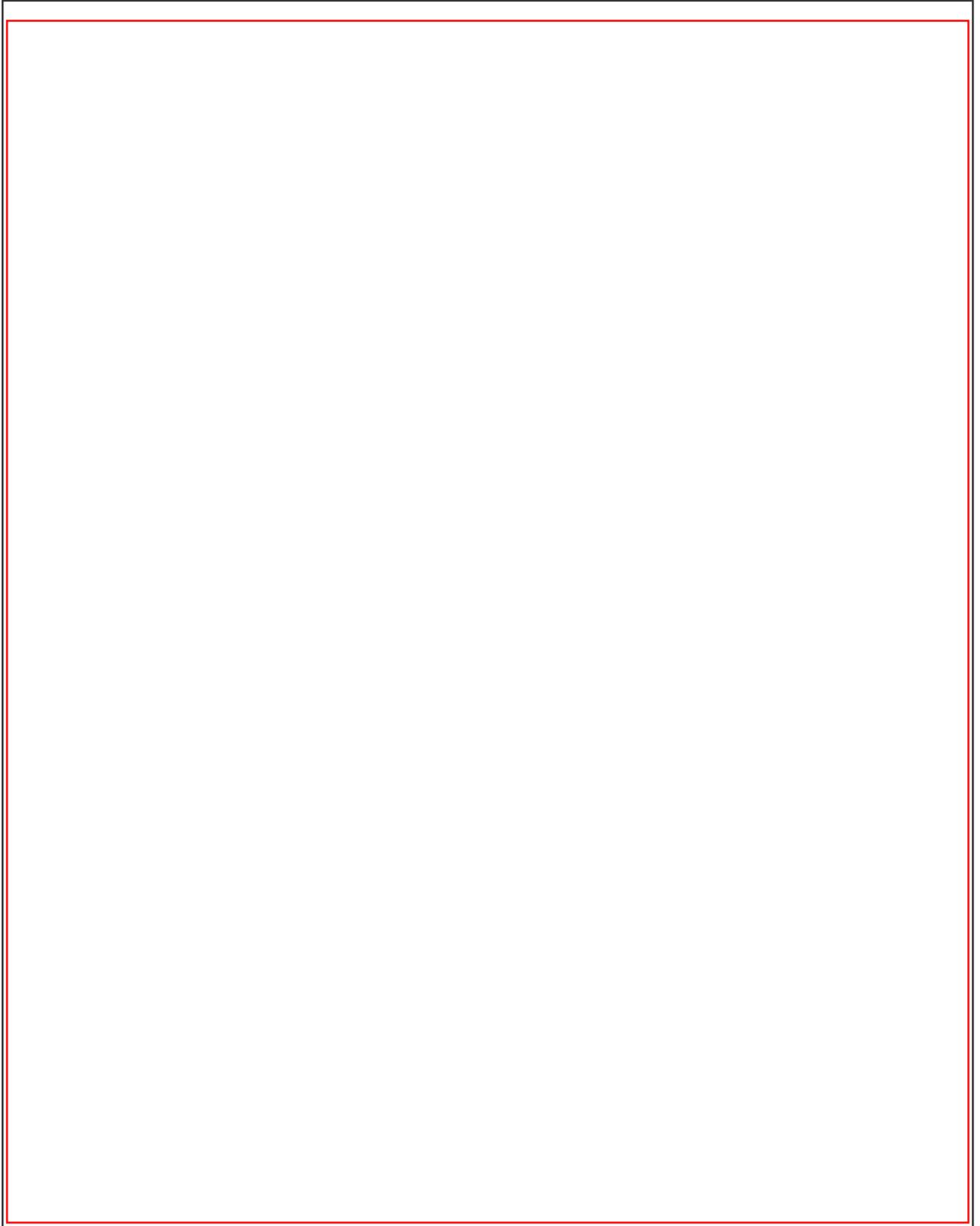
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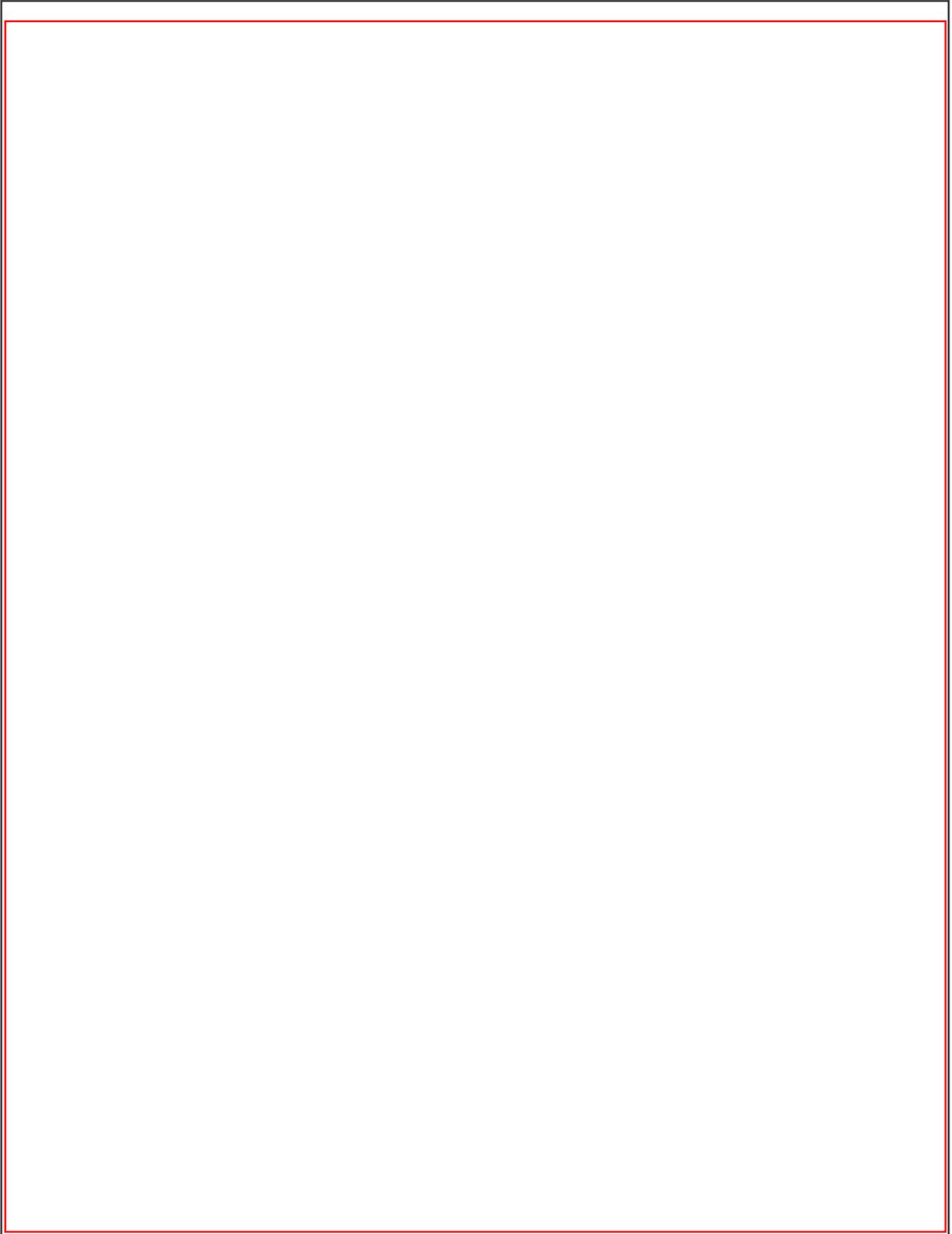
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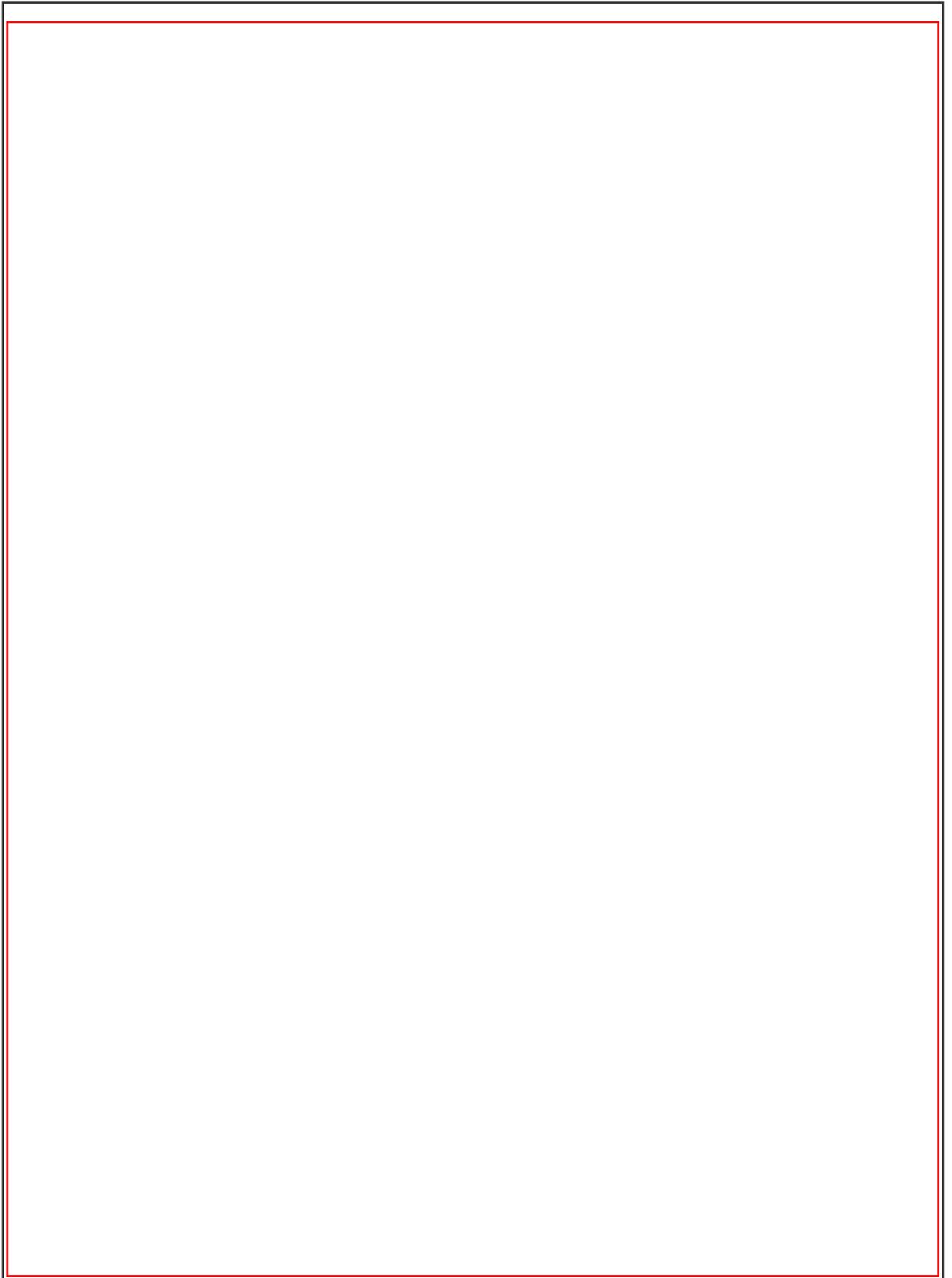
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Corroborating Safety
Studies Summary

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ATTACHMENT 2
RESULTS OF COMPOSITIONAL ANALYSIS OF 7 LOTS OF KIWIBERRY LIQUID
CONCENTRATE

000151

Table A2-1 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 (ppm) ^b	<10	<10	<10	<10	<10	<10	<10	<10
Lead (ppm) ^a	<1	<0.010	0.081	0.031	0.053	0.023	<0.010	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
<i>Escherichia coli</i> (MPN/g)	<3	<3	<3	<3	<3	<3	<3	<3
<i>Salmonella</i> spp.	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Molds (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 ppm

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ATTACHMENT 3
SUMMARY TABLES FOR CORROBORATING SAFETY STUDIES

Table A3-1 Studies Conducted with KiwiBerry Concentrate (PG102)				
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 hematological 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
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
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 hematological, 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

Study Design and/or Strain (Number)	Dosage (Oral)	Duration	Observations Relevant to Safety	Reference
Crl:CD rats (20/sex/group) [15/sex/group were necropsied on postnatal Day 85; 5/sex/group on Day 113]	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
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

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Table A3-1 Studies Conducted with KiwiBerry Concentrate (PG102)

Study Design and/or Strain (Number)	Dosage (Oral)	Duration	Observations Relevant to Safety	Reference
Human				
Randomized, double-blind, placebo-controlled trial in 46 subjects with moderately severe atopic dermatitis	0 or 600 mg/day	42 Days	No significant changes in hematological, 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 <i>et al.</i> , 2005

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

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Table A3-2 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 <i>A. arguta</i> powder [~2,000 or 2,500 mg/kg bw/day] or a 1.0 or 5.0% ethanolic extract of <i>A. arguta</i> 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 <i>et al.</i> , 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 testes 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 <i>et al.</i> , 2003 [Abstract only]
Male rats (10/group)	Hydro-alcoholic extract of <i>A. chinensis</i> 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 <i>et al.</i> , 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 <i>et al.</i> , 2001
Placebo-controlled crossover trial with 25 elite athletes	Kiwi (<i>A. chinensis</i>) 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 <i>et al.</i> , 1990

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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 (<i>A. deliciosa</i> 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 <i>et al.</i> , 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 <i>ex vivo</i> 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 <i>et al.</i> , 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

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SUBMISSION END

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Reference List for Industry Submission, GRN 000215

<i>Pages</i>	<i>Author</i>	<i>Title</i>	<i>Publish Date</i>	<i>Publisher</i>	<i>BIB_Info</i>
000028 - 000035	Boldingh, H.; G.S. Smith; Klages, K.	Seasonal Concentrations of Non-structural Carbohydrates of Five Actinidia Species in Fruit, Leaf and Fine Root Tissue	2000	Annals of Botany	Volume 85, pgs. 469-476
000036 - 000043	Boyes, Stewart; Strubi, Peter; Marsh, Hinga	Sugar and Organic Acid Analysis of Actinidia arguta and Rootstock- Scion Combinations of Actinidia arguta	1996	Lebensm-Wiss. u.- Technol..	Volume 30, pgs. 390-397
000044 - 000050	Klages, Karin; Donnison, Helen; boldingh, Helen; MacRae, Elspeth	myo-Inositol is the major sugar in Actinidia arguta during early fruit development	1998	Aust. J. Plant Physiol	Volume 25, pgs. 61-67
000051 - 000055	Okamoto, Goro; Goto, Shintaro	Juice Constituents in Actinidia arguta Fruits Produced in Shinjo, Okayama	2005	Scientific Reports of the Faculty of Agriculture Okayama University	Volume 94, pgs. 9-13
000056 - 000068	Zhang, Jiasheng; Wang, Baozhen; Li, Pingya; Bingru, Cin Liu	The Nutritional Components of Actinidia	June 1992	NA	Volume 14, Number 2, pgs. 215 - 220

NA- Not applicable

Felix Jr, Fred B*

From: Martin, Robert L
Sent: Monday, April 16, 2007 9:52 AM
To: Felix Jr, Fred B*
Cc: Ramos-Valle, Moraima
Subject: FW: KiwiBerry Concentrate GRAS Notice Addendum
Attachments: Cover Letter 4.12.07.pdf; FCT-S-07-00029.fdf; Efficas Cover Letter Jan 12.07.pdf; WIL Conflict of Interest Letter.pdf; GRN 215 Addendum 4.12.07.pdf

Freddy, can you upload this for GRN 215? Moraima has the hard copies.

Thanks.
Robert L. Martin
301-436-1219

Thanks.
Robert L. Martin
301-436-1219

From: Julie Lindemann [mailto:jlindemann@efficas.com]
Sent: Thursday, April 12, 2007 5:01 PM
To: Martin, Robert L; Ramos-Valle, Moraima
Subject: KiwiBerry Concentrate GRAS Notice Addendum

Dear Dr. Martin,
As a follow-up to our meeting on March 21, 2007, Efficas is submitting an Addendum to the KiwiBerry Concentrate GRAS Notice GRN 215. This includes an expanded discussion of results from component analysis of the ingredient and the parent fruit, *Actinidia arguta*. Also, per your request, a copy of the manuscript submitted to Food and Chemical Toxicology is provided. You will receive tomorrow by Fed Ex three copies of the Addendum and the manuscript. The information is also attached to this email in case that may be helpful.

Please do not hesitate to contact me if you have any questions or concerns.

Thank you.

Best regards,

Julie Lindemann, Ph.D.
Director of Regulatory Affairs
Efficas, Inc.
3260 Blume Dr. #310
Richmond, CA 94806

Tel: 510-669-9496
Mobile: 925-998-1658
FAX: 510-669-9951
FAX: 510-669-995

4/18/2007

Efficas™

Innovations
validated for wellness
and health

April 12, 2007

Robert L. Martin, Ph.D.
Deputy Director
Division of Biotechnology and GRAS Notice Review
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway, HFS-255
College Park, MD, 20740

Re: GRAS Notice for KiwiBerry Concentrate, GRN 215, Amendment

Dear Dr. Martin,

Please find enclosed three copies of an amendment to GRN 215, GRAS Notice for KiwiBerry Concentrate. The amendment contains additional information you and your colleagues requested during our meeting March 21, 2007.

Please do not hesitate to contact me if you require additional information or clarification.

Sincerely,

(b)(6)

Julianne Lindemann, Ph.D.
Director of Regulatory Affairs
Tel: (510) 669-9496
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Elsevier Editorial System(tm) for Food and Chemical Toxicology

Manuscript Draft

Manuscript Number:

Title: A subchronic oral toxicity study of hardy kiwi concentrate in rats

Article Type: Full Length Article

Section/Category:

Keywords: hardy kiwi; Actinidia arguta; rat; subchronic; No Observed Effect Level; toxicity

Corresponding Author: Julianne Lindemann, PhD

Corresponding Author's Institution: Efficas, Inc.

First Author: Melissa Beck, PhD

Order of Authors: Melissa Beck, PhD; Joelle Ibanes, DVM; Michelle Pershing; Mark Nemec; Donald Stump, PhD; Julianne Lindemann, PhD; Dean Stull, PhD

Manuscript Region of Origin:

Abstract:

January 12, 2007

Editors
Food and Chemical Toxicology
Elsevier

Submission: A subchronic oral toxicity study of hardy kiwi concentrate in rats

Dear Editor,

Please find enclosed a scientific manuscript for consideration of publication in Food and Chemical Toxicology. The focus is food safety. The study was designed to evaluate the long-term impact of feeding to rats a cooked concentrate of the hardy kiwi fruit (*Actinidia arguta*), a species of kiwi fruit that is very commonly consumed in Asia but much less commonly in the United States. No previous toxicity studies with this material have been published.

Efficas, Inc., the sponsor of the research, was interested in an objective study to evaluate the safety of the hardy kiwi concentrate on the growth and development of rats, starting at a very early age. The concentrate manufactured from the hardy kiwi fruit, which has a history of consumption in Asia and is compositionally very similar to the common kiwifruit, *Actinidia deliciosa*, was not expected to exhibit overt toxicity, and thus additional endpoints that could detect more subtle adverse impacts in vulnerable animals during development were included in the study. These endpoints included a functional observational battery and femur length.

The findings were that the no observed adverse effect level (NOAEL) was 2,000 mg/kg body weight, the highest dose tested. The results of the study support the safety of hardy kiwi concentrate for human consumption.

The study was conducted under GLP by an independent laboratory, WIL Research, LLC. The sponsor company, Efficas, Inc., which has a financial interest in the potential marketing of the hardy kiwi concentrate as a food ingredient, provided the test article and funding for the study. The sponsor company also provided the technical information on the manufacturing method and composition of the test article. The first 5 authors are affiliated with WIL Research, and Melissa Beck has provided a signed statement that Efficas did not interfere with conduct of the study or interpretation of the results (attached). Dean Stull and J. Lindemann are employees of Efficas, Inc.

I have attached PDF files for the 6 tables and 3 figures.

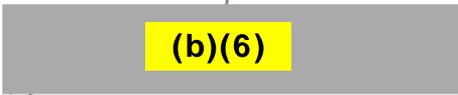
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Regards,


(b)(6)


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Efficas, Inc.
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Tel: (510) 669-9496
Email: jlindemann@efficas.com



November 2, 2006

Joseph F. Borzelleca
Department of Pharmacology
and Toxicology
Medical College of Virginia
Richmond, VA 23298-0613, USA

Ref: Manuscript Submission

Dear Joseph:

Efficas, Inc., provided test article and funded the study:

"A subchronic oral toxicity study of hardy kiwi concentrate in rats."

However, they did not interfere with the conduct of the study or bias reporting of the results by WIL Research Laboratories, LLC.

Sincerely,

(b)(6)

Melissa J. Beck, PhD
Assistant Director, Neuroscience

MJB2/mp

A subchronic oral toxicity study of hardy kiwi concentrate in rats

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Abbreviations: ADP, adenosine diphosphate; ANOVA, one-way analysis of variance; EDTA, ethylenediaminetetraacetic acid; FOB, functional observational battery; GLP, Good Laboratory Practice; GMP, Good Manufacturing Practice; GRAS, Generally Recognized as Safe; HPMC, hydroxypropylmethyl cellulose; Ig, immunoglobulin; IL, interleukin; N, number of animals; NOAEL, no observed adverse effect level; PND, post-natal day; Th1, T-helper cell 1; Th2, increasing T-helper cell 2

Abstract

Background. KiwiBerry Concentrate is a food ingredient produced from hardy kiwi fruit (*Actinidia arguta*).

Objective. To evaluate the potential effects of long-term administration of KiwiBerry Concentrate in a Good Laboratory Practice-compliant subchronic rat toxicity study.

Methods. KiwiBerry Concentrate was administered orally to 160 juvenile male and female Crl:CD(SD) rats during post-natal days 8 to 84. Twenty animals/sex/group were randomized to 1 of 4 groups receiving 0 (control), 500, 1,000, or 2,000 mg/kg body weight/day of KiwiBerry Concentrate. Clinical observations, body weights, food consumption, and indicators of physical development were evaluated, including a functional observational battery (FOB) assessment. Complete necropsies including clinical and gross pathology evaluations were conducted for all animals.

Results. There was no evidence of toxicity at any dose level. Measured parameters were similar between treatment animals and controls. There were no test article-related effects on clinical pathology or organ weights following 77 days of dosing or following the 4-week recovery period. There were no test article-related macroscopic or microscopic changes in any tissues examined at the scheduled necropsies.

Conclusions. The no observed adverse effect level (NOAEL) was 2,000 mg/kg body weight/day. The results of this study support the safety of KiwiBerry Concentrate for human consumption.

Introduction

KiwiBerry Concentrate is a food ingredient produced by concentrating the hot water soluble components of hardy kiwi fruit (*Actinidia arguta*). The fruit also is known by the common names arguta, kokuta, tara vine, baby kiwi, and cocktail or grape kiwi fruit. Hardy kiwi fruit is similar in taste to the common “fuzzy” kiwi fruit, *Actinidia deliciosa*, but is smaller with a non-fuzzy, smooth green skin (Strik and Cahn, 1996). *A. arguta* is cultivated in northern China, Japan, Korea, and Siberia, and is an indigenous plant to these regions. The fruit has a long documented history of human consumption (Dunn, 1911; Darrow and Yerkes, 1937; 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). In addition to its history of cultivation in Asia, the hardy kiwi is now also cultivated in the United States, Canada, France, Germany, Italy, and New Zealand (Strik and Cahn, 1996; Ferguson, 1999).

KiwiBerry Concentrate has been self-affirmed as Generally Recognized as Safe (GRAS), based on compositional similarity of the concentrate to the fuzzy kiwi and the fresh hardy kiwi fruit, and historical consumption of the fresh fruit of both kiwi types, as a food ingredient in a variety of traditional food products such as baked goods, beverages, jams, snack foods, and soft candy. In addition to its use as a food ingredient, increased interest has recently developed regarding the potential beneficial activities of the hardy kiwi. Experiments in mice indicate that specific preparations of KiwiBerry Concentrate may be an active immune system modulator with utility in treating allergic diseases such as atopic dermatitis (Park et al., 2005). The potential toxicity of KiwiBerry Concentrate (referred to as PG102) was investigated in 4 non-Good Laboratory Practice (GLP)-compliant toxicity studies. These studies demonstrated no adverse effects in mice

at doses of up to 150 mg/kg body weight/day for a period of 3 months (PanGenomics Co., Ltd. and Seoul National University (unpublished) a, b), and no adverse effects in rats at doses of up to 1,000 or 300 mg/kg body weight/day for periods of 28 days and 6 months, respectively (PanGenomics Co., Ltd. and Seoul National University (unpublished) c, d). Furthermore, KiwiBerry Concentrate was reported to be non-mutagenic in the Ames assay at a level of up to 5,000 µg/plate, with or without metabolic activation, using *Salmonella typhimurium* (MDS Pharma Services, 2004). Although historical consumption data indicate the hardy kiwi fruit is safe to consume, no published information exists regarding the safety of the water-soluble concentrate. Therefore, a subchronic toxicity study was performed with KiwiBerry Concentrate in juvenile Crl:CD(SD) rats to evaluate the potential for toxicity, with particular emphasis on effects on growth and development.

2. *Materials and Methods*

2.1 *Test Material Production and Characterization*

KiwiBerry Concentrate is produced by solubilizing the hot water soluble components of hardy kiwi fruit, followed by filtration and evaporative concentration. Briefly, the hardy kiwi fruit are harvested, sliced, dried and cooked in 80 to 90°C water to effect nutrient solubilization, and the insoluble components are then removed by filtration. Water is the only solvent used in the process. KiwiBerry Concentrate is composed mainly of carbohydrates (at least 70%), with a minor amount of protein and fat (less than 10% of each), lesser amounts of ash (less than 8%), vitamin C, and several minerals (calcium, magnesium, phosphorus, potassium, and sodium). 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 since they are heat labile, most are at levels much lower than identified in the fresh fruit. The levels of organic acids present in KiwiBerry Concentrate are similar to those present in the fresh fruits (>50 mg/kg). Summaries of the published and analytical values for the various components of KiwiBerry Concentrate, hardy kiwi, and green kiwi are presented in Tables 1 through 3.

<Insert Table 1 here>

<Insert Table 2 here>

<Insert Table 3 here>

Current Good Manufacturing Practices (GMP) are employed during all processing operations. The KiwiBerry Concentrate used in the study was analyzed to confirm test article content, homogeneity (The National Food Laboratory, Inc., Dublin, CA), and stability (Medallion Laboratories, Minneapolis, MN) using standard sampling and analytical techniques. Once received, the test material was stored frozen to preserve its stability.

2.2 *Animals*

Thirty (30) age-matched pregnant female Crl:CD(SD) rats, between gestation days 4 and 7, were obtained from Charles River Laboratories, Inc. (Raleigh, NC). This animal model is recognized as appropriate for juvenile rodent studies and has been shown to be susceptible to the effects of reproductive toxicants. Upon receipt, each female was examined by a qualified technician and uniquely identified by a metal ear tag. During the gestation period, the rats were observed twice daily for mortality and morbidity. All surviving breeder dams were allowed to deliver naturally

and rear their pups to post-natal day (PND) 21. The day parturition was first observed was designated PND 0 for the offspring. After weaning, each pup was uniquely identified by a metal eartag. To reduce variability, litters were culled to 8 pups/litter, 4/sex when possible, on PND 4 using a computerized randomization procedure.

2.3 *Diet and Housing*

Animals were housed throughout the acclimation period and during the study in an environmentally controlled room ($22 \pm 3^{\circ}\text{C}$ and $50 \pm 20\%$ relative humidity). Light timers were calibrated to provide a 12-hour light/12-hour dark photoperiod. Weaned juveniles were housed together by litter until PND 28. Beginning on PND 28, the juvenile rats were individually housed in wire-mesh cages suspended above cage-board. Animals were maintained in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996). The facility (WIL Research Laboratories, LLC) is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. The basal diet (PMI Nutrition International, LLC, Certified Rodent LabDiet[®] 5002) and the reverse osmosis-purified drinking water (delivered by an automatic watering system) were provided *ad libitum* throughout the study.

2.4 *Vehicle and Test Article Preparation*

The vehicle used was 1% (w/v) hydroxypropylmethyl cellulose (HPMC) (Methocel, The Dow Chemical Company, Midland, MI). To prepare the vehicle, the appropriate amount of HPMC powder was quantitatively transferred to and dissolved in a pre-determined volume of deionized water that had been heated to approximately 70°C . After cooling, additional deionized water

was added to achieve the required final volume, and the solution was mixed until uniform using a magnetic stirrer. A sufficient volume of the vehicle was prepared approximately weekly for administration to the control group and for preparation of the test article formulations. Aliquots were prepared for daily dispensation to the control group. The vehicle was mixed throughout preparation, sampling, and dose administration procedures. The test article was supplied as a brown paste (Lot no. SG05-0217-A, Efficas, Inc., Boulder, CO) and formulations were prepared on a weight/volume (test article/vehicle) basis. For the test article-treated groups, the appropriate amount of the test article for each group was weighed into a tared, calibrated glass container. A stir bar and a small amount of vehicle were added, and the contents were mixed. A sufficient volume of the vehicle was added to each container to bring the formulations to the calibration mark. The formulations were homogenized for approximately 15 minutes using a Silverson L4RT homogenizer and then stirred using a magnetic stirrer until a uniform mixture was obtained. Due to foaming, the test article formulations were mixed overnight in a refrigerator. The test article formulations were prepared weekly as single formulations for each dosage level, divided into aliquots for daily dispensation, and stored refrigerated. The test article formulations were stirred continuously throughout the preparation, sampling, and dose administration procedures. Prior to dosing, the test article formulations were visually inspected for homogeneity. Analytical confirmation of homogeneity and stability was conducted concurrently with dosing (National Food Laboratory, Inc., Dublin, CA).

2.5 *Experimental Design*

The study was conducted at WIL Research Laboratories, LLC (Ashland, OH) in accordance with both the U.S. Food and Drug Administration GLP Standards and the *Animal Welfare Act*

Regulations. On or before PND 7 (the day prior to dose administration), all juvenile animals were weighed and examined in detail for physical abnormalities. One male and 1 female pup from each litter were then randomly assigned to 1 of 4 treatment groups. The experimental design for this study consisted of 3 test article-treated groups and 1 control group, each composed of 20 juvenile rats/sex. The selected animals were 8 days old at the initiation of test article administration. Male body weights ranged from 8.3 to 23.5 g and female body weights ranged from 9.7 to 21.1 g on the initial day of test article administration.

2.6 Test Article Administration

The test and vehicle control article formulations were administered by oral gavage using an appropriately-sized stainless steel dosing needle (Popper and Sons, Inc., New Hyde Park, NY) or flexible Teflon[®]-shafted stainless steel ball-tipped dosing cannula (Natume, Japan), as appropriate for the age of the animal, once daily from PND 8 to 84. A dosage volume of 10 mL/kg body weight was used. Individual dosages were based on the most recently recorded body weights to provide the correct mg/kg body weight/day dose. All animals were dosed at approximately the same time each day.

2.7 In-Life Clinical Assessment

All animals were observed twice daily, once in the morning and once in the afternoon, for mortality and moribundity. Detailed physical examinations were recorded on PND 8, 10, 12, 14, 16, 18, 21, 23, 25, and 28, and twice weekly thereafter until euthanasia. In addition, the animals were observed for appearance, behavior, and pharmacotoxic signs approximately 1 hour following dose administration. Individual male and female body weights were recorded on PND

8, 10, 12, 14, 16, 18, 21, 23, 25, and 28, and twice weekly thereafter until euthanasia. Mean body weights and body weight changes were reported for each interval. In addition, cumulative mean body weight changes were reported for the treatment period (PND 8 to 84) and for the post-treatment recovery period (PND 84 to 112). Individual male and female food consumption was measured twice weekly from PND 28 until euthanasia. Food consumption was calculated and reported as g/animal/day and g/kg body weight/day for the corresponding body weight change intervals.

2.8 Developmental Parameters

2.8.1 Balanopreputial Separation

Each male was observed for balanopreputial separation beginning on PND 35 (Korenbroet et al., 1977). Examination of the animal continued daily until balanopreputial separation was complete. The age at which balanopreputial separation was first observed was recorded for each animal. Body weights were recorded at the age of attainment of separation.

2.8.2 Vaginal Perforation

Each female was observed for vaginal perforation beginning on PND 25 (Adams et al., 1985). Examination of the females was continued daily until vaginal patency was present. The age at which the vaginal lumen was first observed to open was recorded for each animal. Body weights were recorded at the age of attainment of patency.

2.9 Functional Observational Battery Assessments

Functional observational battery (FOB) assessments were recorded for all animals during the 10th week of dose administration, when the animals were 74 to 82 days of age. The FOB assessments were conducted prior to dose administration. The FOB is based on previously-developed protocols (Irwin, 1968; Gad, 1982; Moser et al., 1988, 1991; Haggerty, 1989; O'Donoghue, 1989). Testing was performed by the same technicians, whenever possible, without knowledge of the animal's group assignment. The FOB was performed in a sound-attenuated room equipped with a white-noise generator set to operate at 70 ± 10 dB. All animals were observed for the following parameters: *Home cage observations* [posture, convulsions/tremors, feces consistency, biting, palpebral (eyelid) closure]; *Handling Observations* [ease of removal from cage, lacrimation/chromodacryorrhea, piloerection, palpebral closure, eye prominence, red/crusty deposits, ease of handling animal in hand, salivation, fur appearance, respiratory rate/character, mucous membranes/eye/skin color, muscle tone]; *Open Field Observations* (2-minute observation period) [mobility, rearing, convulsions/tremors, grooming bizarre/stereotypic behavior, time to first step (seconds), gait, arousal, urination/defecation, gait score, backing]; *Sensory Observations* [approach response, startle response, pupil response, forelimb extension, air righting reflex, touch response, tail pinch response, eyeblink response, hindlimb extension, olfactory orientation]; *Neuromuscular Observations* [hindlimb extensor strength, hindlimb foot splay, grip strength (hind and forelimb), rotarod performance]; and *Physiological Observations* [catalepsy, body temperature, body weight].

2.10 Clinical Pathology

Blood samples for clinical pathology evaluations (hematology and serum chemistry) were collected from 15 animals/sex/group on PND 85 (1 day following the end of dosing) and from 5

animals/sex/group, when possible, on PND 113 (following the 4-week recovery period). Prior to blood collection, the animals were fasted overnight and then anesthetized with isoflurane. Blood was collected from the vena cava into tubes containing ethylenediaminetetraacetic acid (EDTA) (hematology), sodium citrate (clotting determinations), or no anticoagulant (serum chemistry). The following hematological parameters were evaluated: total leukocyte count, erythrocyte count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, prothrombin time, activated partial thromboplastin time, reticulocyte count (percent and absolute), differential leukocyte count (percent and absolute) (neutrophil, lymphocyte, monocyte, eosinophil, basophil, and large unstained cell).

The following serum chemistry parameters were determined: albumin, total protein, globulin, albumin/globulin ratio, total bilirubin, urea nitrogen, creatinine, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, gamma glutamyltransferase, glucose, total cholesterol, calcium, chloride, phosphorus, potassium, and sodium.

2.11 Gross Pathology

A complete necropsy was conducted on all animals that were found dead or at termination (1 day or 4 weeks following the end of the dosing period). Animals were euthanized by isoflurane inhalation followed by exsanguination. The necropsies included, but were not limited to, examination of the external surface, all orifices, the external surfaces of the brain and spinal cord and the cranial, thoracic, abdominal and pelvic cavities, including viscera. The following tissues and organs were collected and placed in 10% neutral-buffered formalin: adrenal glands, aorta, bone with marrow (sternbrae), brain (forebrain (cerebrum level 1), midbrain (cerebrum level 2),

and hindbrain (cerebrum with pons and medulla)), cervix, coagulating gland, femur, gastrointestinal tract (esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, and rectum), heart, kidneys, liver (sections of 2 lobes), lungs (including bronchi, fixed by inflation with fixative), lymph nodes (mesenteric), mammary gland (females only), ovaries with oviducts, pancreas, peripheral nerve (sciatic), pituitary, prostate, mandibular salivary glands, seminal vesicles, skeletal muscle (rectus femoris), skin (with mammary gland), cervical spinal cord, spleen, vas deferens, thymus, thyroid (with parathyroids, if present), trachea, urinary bladder, uterus with vagina, and gross lesions. Eyes with optic nerve were fixed in Davidson's solution. Testes with epididymides were fixed in Bouin's solution.

2.12 Organ Weights

The following organs were weighed from all animals at the scheduled necropsies (treatment and post-treatment subgroups): adrenal glands, brain, epididymides (total), kidneys, liver, ovaries, pituitary, prostate, seminal vesicles with coagulating glands and accessory fluids, spleen, testes, thymus, uterus (with oviducts and cervix); paired organs were weighed together. Organ to final body weight and organ to brain weight ratios were calculated. The length of the femur was also recorded for animals examined at the scheduled necropsies.

2.13 Microscopic Pathology

After fixation, tissues were trimmed, processed into paraffin blocks, sectioned at 4 to 8 microns, mounted on glass microscope slides and stained with hematoxylin and eosin. Microscopic examination was performed on all tissues listed (except the femur), from all animals (15/sex) in the control and in the 2,000 mg/kg body weight/day treatment subgroups at the scheduled

necropsy on PND 85 and from all animals that were found dead. Microscopic examinations were not performed on surviving animals in the post-treatment subgroup. Missing tissues were identified as not found at necropsy, lost at necropsy, lost during processing, not in the plane of section or other designations as appropriate. A qualified senior veterinary pathologist performed the microscopic examination.

2.14 Statistics

All statistical tests were performed using appropriate computing devices or programs. Analyses were conducted using two-tailed tests (except as noted otherwise) for minimum significance levels of 1 and 5%, comparing each test article-treated group to the control group by sex. Each mean was presented with the standard deviation and the number of animals (N) used to calculate the mean. Body weight, body weight change, food consumption, mean age and weight at attainment of developmental landmarks, continuous FOB, clinical pathology, femur length, and organ weight data were subjected to a parametric one-way analysis of variance (ANOVA) to determine intergroup differences. If the ANOVA revealed statistically significant ($p < 0.05$) intergroup variance, Dunnett's test (Dunnett, 1964) was used to compare the test article-treated groups to the control group. FOB parameters that yielded scalar or descriptive data were analyzed using Fisher's Exact Test (Steel and Torrie, 1980).

3. Results

3.1 Clinical Observations and Survival

Three males, one in each of the control, 500, and 1,000 mg/kg body weight/day groups, were found dead between 50 minutes and 2.5 hours following dose administration on PND 10, 63, and

19, respectively. There were no clinical findings for the unexpected deaths and the only internal macroscopic finding was of lungs that were not fully collapsed for the rat in the 1,000 mg/kg body weight/day group. Although a cause of death could not be determined from the microscopic findings, the occurrence of a death in the control group, combined with the fact that no mortalities occurred in males at 2,000 mg/kg body weight/day or in females at any dosage level, suggests that the deaths of single males at 500 and 1,000 mg/kg body weight/day are not test article-related. One animal in the 500 mg/kg body weight/day group was euthanized and discarded due to a sexing error (initially identified as a female and later determined to be a male). All other males and females survived to the scheduled necropsies on PND 85 or 113. There were no test article-related clinical observations during the detailed physical examinations or 1 hour following dose administration. All clinical findings were noted with similar incidence in the control group, were limited to single animals, were not noted in a dose-related manner, and/or were common findings for laboratory rats of this age and strain.

3.2 *Body Weights*

As shown in Figure 1, no test article-related effects on mean body weights were noted for any of the treatment groups in either males or females. A statistically significant ($p < 0.05$) decrease in mean body weight gain was observed in the 500 mg/kg body weight/day group females for PND 39 to 42 (data not shown); however, because the decrease was not observed at the higher dosage levels, or in both sexes, it was not attributed to the test article. During the post-treatment period, increased mean body weight gains were noted in the 1,000 and 2,000 mg/kg body weight/day group females from PND 91 to 94. The difference from the control group was statistically significant ($p < 0.05$) at 2,000 mg/kg body weight/day. Mean body weights for the 1,000 and

2,000 mg/kg body weight/day groups also were increased (not statistically significant) by up to 9.5 and 12.3% respectively, during the post-treatment period as a result of 3 females in each group that weighed 11 to 23% more than the respective group means; however, these same females weighed 13 to 30% more than the respective group means throughout the treatment period, and there was no increase in mean body weight gain in these groups during the post-treatment period. Therefore, the apparent increases in mean body weights in the 1,000 and 2,000 mg/kg body weight/day groups following the end of the dose administration period were attributed to selection bias and small sample size.

<Insert Figure 1 here>

3.3 *Food Consumption*

As seen in Figures 2 and 3, the average overall food consumption on a g/kg body weight basis (PND 8 to 112) indicates that there were no overall significant differences between treatment groups and controls, nor were any significant differences observed between groups on a g/animal basis (data not shown). Although statistically significant increases ($p < 0.05$ or $p < 0.01$) in food consumption (g/kg body weight/day) were observed in the 2,000 mg/kg body weight/day group females during PND 39 to 42 and in the 500 mg/kg body weight/day group females during PND 87 to 91, they were isolated incidences, did not exhibit time- or dose-related relationships, and similar increases were not observed for the g/animal/day food consumption (data not shown). As a result, they were not attributed to test article administration.

<Insert Figures 2 and 3 here>

3.4 *Developmental Parameters*

3.4.1 *Balanopreputial Separation*

Mean ages of attainment of balanopreputial separation and the respective mean body weights at the age of attainment were unaffected by KiwiBerry Concentrate consumption. The mean ages of attainment of balanopreputial separation were 43.4, 44.3, and 44.8 days in the 500, 1,000, and 2,000 mg/kg body weight/day groups, respectively, compared to 43.6 days in the control group. Mean body weights at the age of attainment were 232.7 g, 233.6 g, and 234.7 g in the same respective groups compared to 231.5 g in the control group. None of the differences from the control group were statistically significant.

3.4.2 *Vaginal Patency*

KiwiBerry Concentrate had no effect on the mean ages of vaginal patency attainment and mean body weights at the age of attainment were unaffected by test article administration. The mean ages of attainment of vaginal patency were 33.3, 32.7, and 33.3 days in the 500, 1,000, and 2,000 mg/kg body weight/day groups, respectively, compared to 32.5 days in the control group. Mean body weights at the age of attainment were 114.9 g, 114.3 g, and 115.0 g in the same respective groups compared to 116.0 g in the control group. None of the differences from the control group were statistically significant.

3.5 *Functional Observational Battery*

There were no observations during any of the tests that indicated treatment-related differences in animal behavior with relation to home cage, handling, open field, sensory, neuromuscular, or physiological activity.

3.6 *Clinical Pathology*

3.6.1 *Hematology*

There were no test article-related effects on hematological parameters (Table 4). The only statistically significant ($p < 0.05$) differences from the control group were lower mean absolute counts of monocytes and leukocytes in the 2,000 mg/kg body weight/day group males on PND 113 (data not shown). As no significant changes were observed at the end of treatment at PND 85, the decreased monocyte and leukocyte counts during the post-treatment evaluation were believed to be due to low animal numbers ($n=5$) at that time-point and were considered to be the result of normal biological variation.

<Insert Table 4 here>

3.6.2 *Serum Chemistry*

As seen in Table 5, there were no test article-related serum chemistry changes. A statistically significant ($p < 0.05$) reduction in the mean cholesterol level was noted in males of the 500 mg/kg body weight/day group compared to the control group on PND 85. In the absence of similar changes in the higher dose groups, this change was considered spurious and not test article-related. Statistically significant ($p < 0.05$ or $p < 0.01$) reductions in mean creatinine levels were

present in all test article-treated female groups compared to the control group on PND 85; however, the range of individual values as well as the distribution within each group were similar to the control group and/or were not dose-related (*i.e.*, control group range: 0.2 to 0.4 mg/dL; 500 mg/kg body weight/day group range: 0.2 to 0.5 mg/dL; 1,000 mg/kg body weight/day group range: 0.2 to 0.4 mg/dL; and 2,000 mg/kg body weight/day group range: 0.1 to 0.5 mg/dL). Therefore, the statistical significance of the finding was considered incidental and the result of normal biological variation.

<Insert Table 5 here>

3.7 *Gross Pathology*

In the 3 males found dead in the control, 500, and 1,000 mg/kg body weight/day groups on PND 10, 63, and 19, respectively, the only internal finding was of lungs that were not fully collapsed for the animal in the 1,000 mg/kg body weight/day group. No relationship to the test article was evident. There were no test article-related macroscopic findings at the scheduled necropsies for the remaining animals.

3.8 *Organ Weights*

There were no test article-related effects on organ weights (Table 6) or femur lengths (data not shown). Lower mean organ weights were present in the 1,000 mg/kg body weight/day group males at the end of the treatment period (absolute pituitary weight and pituitary weight relative to brain weight on PND 85) and at the end of the post-treatment period (absolute kidney weights on PND 113). The differences from the control group were statistically significant ($p < 0.05$ or $p < 0.01$); however, these changes were not considered test article-related given the lack of a dose-

or time-related effect. Lower mean uterus/cervix/oviduct weights (absolute and relative to brain and final body weights) and lower mean kidney weights relative to final body weights were present in the 1,000 and 2,000 mg/kg body weight/day group females at the end of the post-treatment period (PND 113) (data not shown). The differences from the control group were statistically significant ($p < 0.05$ or $p < 0.01$); however, in the absence of any similar change at the end of the treatment period, these lower weights were considered the results of biological variation and were not considered test article-related.

<Insert Table 6 here>

3.9 *Microscopic Pathology*

In the animals that died during the study, none had any microscopic findings that could explain their deaths, and the causes of death were classified as undetermined. Based on the lack of a dose-response relationship, these deaths were not considered test article-related. There were no test article-related microscopic changes in the 2,000 mg/kg body weight/day group males and females euthanized at the end of the treatment period. One female in the 2,000 mg/kg body weight/day group had a mammary gland adenocarcinoma. Although such tumors are extremely rare in animals of that age (12 weeks old), in the absence of any other proliferative changes in the mammary gland of other females in the same dose group, this lesion was considered incidental and unrelated to test article administration. All other microscopic changes were determined to be consistent with normal background lesions in clinically normal rats of the strain and age used in this study.

4. Discussion

Following 77 days of KiwiBerry Concentrate consumption, there were no test article-related mortalities, clinical observations, or effects on mean body weight gain, body weight, food consumption, physical developmental parameters (balanopreputial separation and vaginal patency) or FOB performance (home cage, handling, open field, sensory, neuromuscular and physiological observations) at any dose level during the treatment period (PND 8 to 84). There were no test article-related macroscopic or microscopic (treatment subset only) findings, nor were there any toxicologically significant effects on hematology and serum chemistry parameters, organ weights, or femur lengths in males or females selected for necropsy 1 day following the last dose (15/sex/group) or following a 4-week non-dosing (recovery) period (5/sex/group, when possible). Although significant differences in a variety of toxicological endpoints were observed in some test animals relative to controls, they were considered incidental as the effects were either not consistently observed between sexes, or no dose or temporal relationships in the incidence or severity of effects were observed. Therefore, the no observed adverse effect level (NOAEL) for KiwiBerry Concentrate is considered to be 2,000 mg/kg body weight/day, the highest dose tested, when administered orally to juvenile rats from PND 8 to 84. The results of this study support previous findings of toxicity studies of hardy kiwi concentrate that employed doses of up to 1,000 mg/kg body weight/day for 28 days and up to 150 mg/kg body weight/day in rats and mice, respectively.

Both the hardy kiwi and the fuzzy kiwi have enjoyed years of safe consumption by humans. The recognized differences between hardy kiwi and the common fuzzy kiwi are primarily morphological (Ferguson, 1991; Strik and Cahn, 1996). Slight compositional differences in leaf

flavonoids and relative proportions of common fruit sugars and organic acids occur (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 (USDA, 2005) and the fruits are qualitatively similar. The hardy kiwi ingredient is concentrated from the dried fruit as a water soluble extract at a ratio of 25:1, in a manner such that its constituents remain in proportion to that in the whole fruit, which gives credence to its safety. In addition to the historical consumption of hardy kiwi and its compositional similarity to the common fuzzy kiwi, the safety of the hardy kiwi-derived ingredient is further substantiated by the fact that all of its components [*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. Clinical trials using whole kiwi fruit or ingredients derived thereof indicate that kiwi is well tolerated (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* (golden kiwi) provided to athletes in quantities as high as 1.2 L/person was reported to have no significant effect on heart rate, blood pressure, or electrocardiogram readings and was not associated with any adverse events (Di et al., 1990).

Aside from the use of kiwi fruit in nutritional settings, the potential beneficial effects of the fruit have gained recent interest. As kiwi fruit is high in vitamin C, vitamin E, and polyphenols, Duttaroy and Jorgensen (2004) suggested that the fruit might be cardioprotective. The results of a small, randomized cross-over study (30 volunteers) by Duttaroy and Jorgensen (2004) demonstrated that the consumption of 2 or 3 kiwi fruit per day for 28 days reduced platelet

aggregation response to collagen and adenosine diphosphate (ADP) by 18% compared to controls ($p < 0.05$). The authors also observed a significant ($p < 0.05$) 15% reduction in blood triglyceride levels. Other researchers have investigated the potential immune-modulating properties of the hardy kiwi for therapy in various allergy-mediated diseases. Park et al. (2005) demonstrated that the oral administration of hardy kiwi concentrate to mice could significantly decrease T-helper cell 2 (Th2) cytokine production, while increasing T-helper cell 1 (Th1) production. The alteration in balance between Th1 and Th2 cytokines also was accompanied by decreased plasma levels of immunoglobulin (Ig)-E and IgG1, increased plasma levels of Ig2a and decreased production of IgE-producing B cells and interleukin (IL)-4-producing T cells. Mraz et al. (2006) reported that a kiwi fruit concentrate prepared from hardy kiwi had a positive influence on several parameters of skin health in human subjects with atopic dermatitis, including skin appearance. In this placebo-controlled, randomized, double-blind study with 51 subjects, a statistically significant benefit of the kiwi concentrate was evident while subjects continued their topical steroid use, suggesting the potential of hardy kiwi concentrate to provide adjunctive, dietary management of this allergic condition.

Kiwi allergy is being observed with increasing frequency in the western population (Fiocchi et al., 2004). Symptoms of kiwi allergy range from localized oral irritation and swelling to cases of anaphylactic shock. Kiwi allergy is believed to be IgE-mediated (Lucas et al., 2003) and although specific studies documenting the allergenicity of the hardy kiwi are lacking, it is likely that some individuals will show immune reactivity to the fruit. Evidence for this is suggested by Chen et al. who isolated IgE from the sera of kiwi fruit-allergic subjects and showed cross-reactivity of the antibody to various proteins extracted from the hardy, golden, and fuzzy kiwi fruit varieties (Chen et al., 2006). However, as there is evidence to suggest that kiwi allergens are

heat labile and thus likely lose their immunoreactivity to IgE following steam processing (Fiocchi et al., 2004), there is a significant probability that the hardy kiwi fruit concentrate will lack allergenic potential.

This has been the first GLP study to generate controlled animal toxicity data in relation to the safety of long-term consumption of KiwiBerry Concentrate. The results of this study strongly support the safety of KiwiBerry Concentrate as a dietary ingredient for human consumption. The NOAEL for KiwiBerry Concentrate in juvenile male and female Crl:CD(SD) rats was determined to be 2,000 mg/kg body weight/day.

Conflict of Interest Statement

This work was funded by Efficas, Inc., Boulder, CO., which has a commercial interest in the development of the KiwiBerry Concentrate. Two authors are employees of Efficas; Dean Stull and Julianne Lindemann.

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Fig. 1. Body weights of male and female rats given daily doses of hardy kiwi concentrate at the indicated doses by gavage for 77 days (n=20) followed by a 4-week recovery period (n=5). Data represents the mean of each group +/- S.D. of mean.

Fig 2. Food consumption (g/kg body weight/day) of male rats given daily doses of hardy kiwi concentrate at the indicated doses by gavage for 77 days (n=20) followed by a 4-week recovery period (n=5) beginning at PND 85. Data represents the mean of each group +/- S.D. of mean.

Fig. 3. Food consumption (g/kg body weight/day) of female rats given daily doses of hardy kiwi concentrate at the indicated doses by gavage for 77 days (n=20) followed by a 4-week recovery period (n=5). Data represents the mean of each group +/- S.D. of mean. *p<0.05; **p<0.01.

Table 1
Proximate analysis of KiwiBerry liquid concentrate in comparison to values for *Actinidia arguta* and *Actinidia deliciosa* fruit^a

Parameter	KiwiBerry Concentrate	<i>Actinidia arguta</i>		<i>Actinidia deliciosa</i>
	Analytical Value ^b	Published Value ^c	Analytical Value ^d	Analytical Value ^e
Moisture (%)	33.28 ± 6.1	NA	76.82	83.3
Ash (%)	4.64 ± 0.5	4.44	3.28	5.27
Protein (%)	5.91 ± 0.58	6.85 ± 0.13	6.17	6.65
Fat (%)	4.32 ± 2.14	6.62 to 6.69 ^f	7.38	11.08
Carbohydrate (%)	85.75 ± 2.83	NR	83.18	77.13
Total organic acids (mg/g)	NR	40	NR	NR
Calories (per 100 g)	399.7 ± 12.98	NR	422.78	437.13

^a All measures are expressed on a dry weight basis except for moisture.

^b Mean ± standard deviation of 7 independent lots of manufactured concentrate. Raw material was Oregon-sourced fruit from 2 years harvest.

^c From Zhang *et al.*, 1992. Values are the average of 3 measurements.

^d Fruit was obtained from Oregon. One lot of fruit was tested.

^e Fruit was obtained from a local grocery store. One lot of fruit was tested.

^f Range of pure (6.62%) to crude (6.69%) fat.

NA: not applicable, NR: not reported.

Table 2
Carbohydrate, organic acid and mineral components of KiwiBerry liquid concentrate in comparison to published and analytical values for *Actinidia arguta* and *Actinidia deliciosa* fruit^a

Carbohydrate Component	KiwiBerry Liquid Concentrate ^b (Mean \pm SD)	<i>Actinidia arguta</i>		<i>Actinidia deliciosa</i>	
		Published Values ^c	Analytical Value ^d	Published Values ^c	Analytical Value ^e
Carbohydrate Component (g/100 g)					
Sugars	55.06 \pm 3.44	23.3 to 46.2	23.99	13.4 to 38.8	52.8
Starch	0.13 \pm 0.01	DNR	0.45	DNR	0.52
Fiber	4.88 \pm 1.20	DNR	25.02	DNR	13.17
Sugars (g/100 g)					
Fructose	26.71 \pm 2.13	5.5 to 8.5	10.05	5 to 16.5	52.8
Glucose	23.06 \pm 1.62	5.0 to 8.5	11.09	4.7 to 12.5	0.52
Inositol	4.99 \pm 0.42	1.4 to 2.5	2.85	0.8	13.17
Sucrose ^f	0.30 \pm 0.35	2.5 to 27.5	0.00	2.7 to 2.8	
Organic Acids					
Citric acid (mg/g)	74.41 \pm 8.67	60 to 60.9	35.33	33.9 to 51	38.3
D-Malic acid (mg/g)	15.96 \pm 4.02	11.5 to 13.15	22.86	5 to 13	29.4
Quinic acid (mg/g)	37.67 \pm 4.85	25.95 to 75.5	21.18	32.5 to 41.8	44.05
Vitamin C (ppm)	12.53 \pm 20.83	DNR	149.70	DNR	452.50
Minerals					
Calcium (ppm)	1,267.9 \pm 365	2,160	3,623	1,210	2,455
Magnesium (ppm)	1,131.9 \pm 241	930	906	800	1,018
Phosphorus (ppm)	1,993 \pm 527	NI	2,066	NI	3,180
Potassium (ppm)	21,204 \pm 1,062	13,840	11,260	12,710	22,215
Sodium (ppm)	338 \pm 140	NI	25.02	NI	77.84

^a All measures are expressed on a dry weight basis except for moisture.

^b Mean \pm standard deviation of 7 independent lots of manufactured concentrate. Raw material was Oregon-sourced fruit from 2 years harvest.

^c Literature values reported for ripe fruit at harvest on a wet basis (Zhang *et al.*, 1992; Boyes *et al.*, 1997b; Klages *et al.*, 1998; Boldingh *et al.*, 2000, Okamoto and Goto, 2005). Calculations were made when necessary to standardize units.

^d Fruit was obtained from Oregon. One lot of fruit was tested.

^e Fruit was obtained from a local grocery store. One lot of fruit was tested.

^f 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 manufacture of KiwiBerry Concentrate.

DNR: Did not research this topic; NI: not identified.

Table 3
Phenolic components of KiwiBerry liquid concentrate in comparison to *Actinidia arguta* and *Actinidia deliciosa* fruit^a

Phenolic Component	KiwiBerry Liquid Concentrate ^b		<i>Actinidia arguta</i> ^c	<i>Actinidia deliciosa</i> ^d
	Mean \pm SD	Range		
Flavonoids (ppm)				
Quercetin	62.38 \pm 22.57	21.5 to 82.4	36.07	16.71
Isorhamnetin	25.23 \pm 3.22	ND to 31.4	ND	15.87
Kaempferol	23.74 \pm 3.07	ND to 27.7	ND	12.1
Anthocyanins (ppm)				
Cyanidin	1.13 ^e	ND to 1.13	130.07	ND
Delphinidin	0.20 \pm 0.07	ND to 0.32	9.15	0.18
Malvidin	5.9 \pm 5.89	ND to 15.06	11.22	11.68
Pelargonidin	ND	ND	0.69	ND
Peonidin	0.18 \pm 0.14	ND to 0.35	0.99	ND
Catechins	ND	ND	ND	Not tested

^a All measures are expressed on a dry weight basis.

^b Mean \pm standard deviation of 7 independent lots of manufactured concentrate. Raw material was Oregon-sourced fruit from 2 years harvest.

^c Fruit was obtained from Oregon. One lot of fruit was tested.

^d Fruit was obtained from a local grocery store. One lot of fruit was tested.

^e Cyanidin detected in only 1 of 7 lots tested.

ND: not detected.

Table 4
Summary of hematology values: (a) Males, (b) Females

Analysis PND 85	Group	0 mg/kg/day	500 mg/kg bw/day	1,000 mg/kg bw/day	2,000 mg/kg bw/day
(a) Males					
White cells (thous/uL)	Mean	13.14	13.18	14.01	12.16
	S.D.	2.566	2.956	4.177	1.777
Red cells (mil/uL)	Mean	8.19	8.28	8.32	8.44
	S.D.	0.407	0.374	0.439	0.438
Hemoglobin (g/dL)	Mean	15.9	16.2	16.3	16.4
	S.D.	0.50	0.61	0.74	0.78
Hematocrit (%)	Mean	45.8	47.0	47.2	48.0
	S.D.	1.87	2.92	2.97	2.98
MCV (fL)	Mean	55.9	56.8	56.7	56.9
	S.D.	1.87	2.40	1.49	1.64
MCH (pg)	Mean	19.4	19.6	19.6	19.5
	S.D.	0.79	0.56	0.48	0.33
MCHC (g/dL)	Mean	34.7	34.4	34.6	34.2
	S.D.	0.85	1.03	0.74	0.98
Platelet (thous/uL)	Mean	1171.	1170.	1114.	1126.
	S.D.	141.3	139.9	146.9	186.5
ProTime (seconds)	Mean	15.7	17.0	16.1	16.2
	S.D.	1.43	1.47	1.36	2.25
APTT (seconds)	Mean	23.8	23.6	23.4	23.1
	S.D.	2.35	2.28	2.42	3.52
Reticulocyte (Retic) (%)	Mean	2.1	2.2	2.2	2.1
	S.D.	0.37	0.36	0.25	0.42
Retic absolute (thous/uL)	Mean	172.2	182.3	184.7	177.4
	S.D.	26.97	30.42	25.22	36.39
Neutrophil (Neu) (%)	Mean	9.8	8.2	8.4	10.1
	S.D.	4.98	2.72	2.21	4.05
Lymphocyte (Lymph) (%)	Mean	85.9	87.1	87.2	85.5
	S.D.	5.37	3.64	2.52	4.01
Monocyte (Mono) (%)	Mean	1.7	2.0	1.7	1.8
	S.D.	0.51	0.78	0.56	0.51
Eosinophil (Eos) (%)	Mean	0.8	0.8	0.7	0.8
	S.D.	0.23	0.35	0.30	0.40
Basophil (Baso) (%)	Mean	0.4	0.5	0.4	0.4
	S.D.	0.09	0.27	0.15	0.12
Lg unstain cell (%)	Mean	1.5	1.4	1.6	1.4
	S.D.	0.37	0.59	0.52	0.55
Neu absolute (thous/uL)	Mean	1.35	1.06	1.13	1.20
	S.D.	0.934	0.344	0.283	0.437
Lymph absolute (thous/uL)	Mean	11.22	11.50	12.27	10.42
	S.D.	1.882	2.794	3.830	1.741

Analysis PND 85	Group	0 mg/kg/day	500 mg/kg bw/day	1,000 mg/kg bw/day	2,000 mg/kg bw/day
Mono absolute (thous/uL)	Mean	0.23	0.27	0.23	0.22
	S.D.	0.100	0.103	0.080	0.075
Eos absolute (thous/uL)	Mean	0.10	0.10	0.09	0.09
	S.D.	0.044	0.043	0.033	0.051
Baso absolute (thous/uL)	Mean	0.05	0.06	0.06	0.05
	S.D.	0.019	0.061	0.040	0.017
Lg unstain cell absolute (thous/uL)	Mean	0.19	0.19	0.23	0.18
	S.D.	0.062	0.072	0.111	0.082
(b) Females					
White cells (thous/uL)	Mean	8.27	9.97	10.10	8.92
	S.D.	3.887	2.502	2.180	1.868
Red cells (mil/uL)	Mean	7.97	7.86	7.94	7.97
	S.D.	0.408	0.418	0.458	0.322
Hemoglobin (g/dL)	Mean	15.5	15.3	15.5	15.7
	S.D.	0.72	0.86	0.94	0.56
Hematocrit (%)	Mean	44.3	42.9	43.7	44.4
	S.D.	2.60	2.57	2.93	2.09
MCV (fl)	Mean	55.5	54.6	55.1	55.7
	S.D.	1.37	1.64	1.49	1.37
MCH (pg)	Mean	19.5	19.4	19.6	19.7
	S.D.	0.48	0.43	0.49	0.52
MCHC (g/dl)	Mean	35.1	35.6	35.5	35.4
	S.D.	0.91	0.54	0.62	0.97
Platelet (thous/uL)	Mean	1171.	1106.	1172.	1191.
	S.D.	137.7	159.7	166.9	143.0
ProTime (seconds)	Mean	14.3	14.0	14.3	14.1
	S.D.	0.76	0.95	0.74	0.85
APTT (seconds)	Mean	17.2	17.3	17.3	17.0
	S.D.	1.66	2.81	2.50	2.09
Reticulocyte (%)	Mean	1.9	1.8	1.9	2.1
	S.D.	0.32	0.43	0.63	0.49
Retic absolute (thous/uL)	Mean	153.9	141.8	149.4	170.0
	S.D.	26.31	33.14	52.26	36.06
Neutrophil (%)	Mean	9.4	7.8	8.0	10.8
	S.D.	4.06	3.40	3.16	5.64
Lymphocyte (%)	Mean	86.2	87.8	87.7	84.9
	S.D.	4.63	3.23	3.79	5.86
Monocyte (%)	Mean	1.7	1.7	1.7	1.6
	S.D.	0.54	0.60	0.71	0.40
Eosinophil (%)	Mean	0.7	0.8	0.7	0.9
	S.D.	0.37	0.29	0.40	0.32
Basophil (%)	Mean	0.4	0.4	0.4	0.4
	S.D.	0.21	0.26	0.11	0.14

Analysis PND 85	Group	0 mg/kg/day	500 mg/kg bw/day	1,000 mg/kg bw/day	2,000 mg/kg bw/day
Lg unstain cell (%)	Mean	1.7	1.5	1.5	1.5
	S.D.	0.53	0.40	0.34	0.36
Neu absolute (thous/uL)	Mean	0.71	0.74	0.78	0.92
	S.D.	0.317	0.304	0.306	0.402
Lymph absolute (thous/uL)	Mean	7.21	8.79	8.89	7.61
	S.D.	3.677	2.400	2.133	1.875
Mono absolute (thous/uL)	Mean	0.13	0.17	0.17	0.14
	S.D.	0.055	0.075	0.068	0.043
Eos absolute (thous/uL)	Mean	0.05	0.08	0.07	0.08
	S.D.	0.020	0.034	0.039	0.029
Baso absolute (thous/uL)	Mean	0.03	0.05	0.04	0.04
	S.D.	0.021	0.027	0.013	0.014
Lg unstain cell absolute (thous/uL)	Mean	0.13	0.15	0.15	0.13
	S.D.	0.057	0.059	0.048	0.049

APTT = activated partial thromboplastin time, bw = body weight, fL = femtoliters, g/dL = grams/deciliter, Lg unstain cell = Large unstained cell, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, MCV = mean corpuscular volume, mil/uL = millions/microliter, pg = picograms, PND = post-natal day, ProTime = prothrombin time, thous/uL = thousands/microliter uL = thousands/microliter

No significant differences between the treatment groups and the control group

Table 5
Summary of serum chemistry values: (a) Males and (b) Females

Analysis PND 85	Group:	0 mg/kg bw/day	500 mg/kg bw/day	1,000 mg/kg bw/day	2,000 mg/kg bw/day
(a) Males					
Albumin (g/dL)	Mean	4.2	4.2	4.2	4.3
	S.D.	0.20	0.37	0.26	0.31
Total protein (g/dL)	Mean	6.6	6.8	6.7	6.8
	S.D.	0.40	0.63	0.51	0.57
A/G ratio	Mean	1.74	1.62	1.71	1.76
	S.D.	0.185	0.170	0.137	0.234
Total bili (mg/dL)	Mean	0.1	0.1	0.1	0.1
	S.D.	0.03	0.03	0.03	0.03
Globulin (g/dL)	Mean	2.4	2.6	2.5	2.5
	S.D.	0.28	0.36	0.29	0.35
Urea nitrogen (mg/dL)	Mean	13.6	13.4	14.0	14.4
	S.D.	1.66	1.62	2.35	2.20
Creatinine (mg/dL)	Mean	0.3	0.3	0.3	0.3
	S.D.	0.06	0.11	0.07	0.11
AlkalinePhos'tse (U/L)	Mean	134.	134.	137.	137.
	S.D.	27.1	34.1	17.9	38.2
Alanine transfer (U/L)	Mean	33.	34.	34.	38.
	S.D.	6.0	8.4	7.0	10.0
Aspartattransfer (U/L)	Mean	71.	78.	76.	80.
	S.D.	9.8	12.2	9.1	14.4
GlutamylTransfer (U/L)	Mean	0.6	0.6	0.6	0.7
	S.D.	0.07	0.13	0.13	0.10
Glucose (mg/dL)	Mean	156.	158.	157.	161.
	S.D.	27.6	30.4	19.2	29.5
Cholesterol (mg/dL)	Mean	59.	46.*	59.	55.
	S.D.	13.2	10.2	14.2	15.3
Calcium (mg/dL)	Mean	11.4	11.6	11.5	11.7
	S.D.	0.62	0.82	0.89	0.79
Chloride (meq/L)	Mean	102.	102.	103.	102.
	S.D.	2.0	1.6	2.2	2.6
Phosphorus (mg/dL)	Mean	9.4	10.0	9.7	10.3
	S.D.	1.05	1.72	1.88	1.67
Potassium (meq/L)	Mean	5.10	5.39	5.04	5.57
	S.D.	1.087	1.399	0.965	0.909
Sodium (meq/L)	Mean	146.	148.	149.	149.
	S.D.	2.0	3.8	3.9	2.7
(b) Females					
Albumin (g/dL)	Mean	4.8	4.6	4.6	4.8
	S.D.	0.49	0.38	0.39	0.51

Analysis PND 85	Group:	0 mg/kg bw/day	500 mg/kg bw/day	1,000 mg/kg bw/day	2,000 mg/kg bw/day
Total protein (g/dL)	Mean	7.4	7.0	7.1	7.2
	S.D.	0.60	0.49	0.72	0.52
A/G ratio	Mean	1.86	1.95	1.84	1.93
	S.D.	0.240	0.248	0.198	0.245
Total bili (mg/dL)	Mean	0.1	0.1	0.1	0.1
	S.D.	0.05	0.03	0.05	0.05
Globulin (g/dL)	Mean	2.6	2.4	2.5	2.5
	S.D.	0.27	0.24	0.38	0.18
Urea nitrogen (mg/dL)	Mean	15.7	14.4	14.5	15.0
	S.D.	2.37	2.28	1.38	2.24
Creatinine (mg/dL)	Mean	0.4	0.3*	0.3**	0.3*
	S.D.	0.04	0.09	0.07	0.06
AlkalinePhos'tse (U/L)	Mean	87.	80.	91.	82.
	S.D.	17.3	14.7	24.6	18.0
Alanine transfer (U/L)	Mean	40.	29.	36.	33.
	S.D.	13.3	7.2	14.8	15.2
AspartatTransfer (U/L)	Mean	90.	78.	83.	78.
	S.D.	26.6	9.0	13.3	16.1
GlutamylTransfer (U/L)	Mean	1.2	0.9	1.3	0.9
	S.D.	0.67	0.66	0.73	0.59
Glucose (mg/dL)	Mean	133.	140.	137.	145.
	S.D.	24.8	19.2	13.6	24.1
Cholesterol (mg/dL)	Mean	78.	62.	72.	70.
	S.D.	19.5	11.0	16.9	12.7
Calcium (mg/dL)	Mean	11.6	11.3	11.7	11.8
	S.D.	0.69	0.67	1.08	0.77
Chloride (meq/L)	Mean	103.	103.	104.	102.
	S.D.	1.4	1.9	1.7	1.9
Phosphorus (mg/dL)	Mean	10.6	9.5	10.1	10.3
	S.D.	2.20	1.49	1.43	1.49
Potassium (meq/L)	Mean	5.98	5.42	6.26	6.16
	S.D.	1.014	1.099	1.522	1.316
Sodium (meq/L)	Mean	147.	146.	147.	146.
	S.D.	3.2	2.7	3.4	2.1

A/G Ratio = albumin/globulin ratio, Alanine Transfer = alanine aminotransferase, AlkalinePhos'tse = alkaline phosphatase, AspartatTransfer = aspartate aminotransferase, bw = body weight, Cholesterol =total cholesterol, g/dL = grams/deciliter, GlutamylTransfer = gamma glutamyltransferase, meq/L = milliequivalents/liter, mg/dL = milligrams/deciliter, mil/uL = millions/microliter, Total Bili = total bilirubin, U/L = international unit/liter

* = Significantly different from the control group at 0.05 using Dunnett's test

** = Significantly different from the control group at 0.01 using Dunnett's test

Table 6
Organ weights relative to final body weights [g/100 g] and brain weights [g/100 g]

PND 85	Group	0 mg/kg bw/day	500 mg/kg bw/day	1,000 mg/kg bw/day	2,000 mg/kg bw/day
	Mean (S.D.)				
(a) Males					
Final Body wt (g)		453. (33.0)	453. (35.6)	452. (39.4)	450. (80.1)
Final Brain wt (g)		1.99 (0.108)	1.97 (0.086)	1.99 (0.125)	1.97 (0.146)
Brain	Rel Body	0.441 (0.029)	0.438 (0.038)	0.443 (0.043)	0.453 (0.092)
	Rel Brain	--	--	--	--
Liver	Rel Body	3.022 (0.230)	3.169 (0.207)	3.194 (0.290)	3.140 (0.162)
	Rel Brain	687.361 (57.793)	728.600 (84.580)	728.189 (108.889)	717.064 (137.770)
Kidneys	Rel Body	0.708 (0.056)	0.716 (0.062)	0.692 (0.049)	0.716 (0.082)
	Rel Brain	160.927 (13.521)	164.350 (18.618)	156.860 (11.438)	160.886 (18.493)
Spleen	Rel Body	0.181 (0.023)	0.182 (0.022)	0.188 (0.021)	0.179 (0.027)
	Rel Brain	41.078 (5.934)	41.577 (4.774)	42.849 (6.610)	40.350 (7.334)
Sem Ves/Cg/Fluid	Rel Body	0.382 (0.062)	0.355 (0.064)	0.356 (0.063)	0.375 (0.093)
	Rel Brain	87.145 (15.290)	81.665 (16.941)	80.356 (13.439)	82.645 (12.045)
Prostate	Rel Body	0.175 (0.044)	0.165 (0.037)	0.159 (0.032)	0.176 (0.041)
	Rel Brain	39.755 (10.290)	37.516 (8.267)	36.097 (6.950)	38.959 (5.684)
Epididymides	Rel Body	0.268 (0.023)	0.257 (0.030)	0.255 (0.022)	0.275 (0.064)
	Rel Brain	60.990 (6.456)	58.684 (5.452)	57.862 (4.771)	60.502 (5.028)
Testes	Rel Body	0.808 (0.073)	0.813 (0.073)	0.791 (0.079)	0.847 (0.187)
	Rel Brain	183.821 (18.720)	186.143 (18.739)	179.239 (16.331)	187.360 (17.482)
Thymus	Rel Body	0.128 (0.022)	0.134 (0.016)	0.134 (0.033)	0.126 (0.026)
	Rel Brain	28.970 (4.894)	30.643 (3.966)	30.441 (8.101)	28.303 (6.687)
Adrenal Glands	Rel Body	0.017 (0.003)	0.017 (0.002)	0.017 (0.002)	0.016 (0.003)
	Rel Brain	3.851 (0.634)	3.834 (0.524)	3.804 (0.437)	3.590 (0.712)
Pituitary	Rel Body	0.003 (0.001)	0.003 (0.001)	0.003 (0.001)	0.003 (0.001)
	Rel Brain	0.791 (0.101)	0.712 (0.131)	0.645 (0.123)**	0.728 (0.116)
(b) Females					
Final Body wt (g)	Rel Body	259. (21.1)	245. (25.9)	247. (22.6)	246. (23.9)

PND 85	Group Mean (S.D.)	0 mg/kg bw/day	500 mg/kg bw/day	1,000 mg/kg bw/day	2,000 mg/kg bw/day
Final Brain wt (g)	Rel Brain	1.83 (0.140)	1.80 (0.116)	1.83 (0.088)	1.78 (0.096)
Brain	Rel Body	0.712 (0.072)	0.739 (0.081)	0.744 (0.072)	0.727 (0.066)
	Rel Brain	--	--	--	--
Liver	Rel Body	3.023 (0.192)	3.029 (0.214)	2.963 (0.234)	3.036 (0.263)
	Rel Brain	428.400 (46.262)	414.020 (50.835)	399.765 (30.361)	418.116 (22.794)
Kidneys	Rel Body	0.729 (0.037)	0.750 (0.075)	0.770 (0.065)	0.747 (0.074)
	Rel Brain	103.344 (10.526)	102.043 (10.645)	103.867 (7.876)	102.786 (6.657)
Spleen	Rel Body	0.211 (0.034)	0.212 (0.035)	0.227 (0.026)	0.208 (0.021)
	Rel Brain	29.999 (5.775)	28.927 (4.856)	30.940 (5.754)	28.803 (3.339)
Uterus/CX/OD	Rel Body	0.232 (0.065)	0.245 (0.080)	0.243 (0.059)	0.216 (0.064)
	Rel Brain	32.742 (9.184)	33.529 (11.576)	32.737 (7.855)	29.614 (8.170)
Ovaries	Rel Body	0.040 (0.009)	0.041 (0.005)	0.041 (0.005)	0.041 (0.010)
	Rel Brain	5.650 (1.119)	5.642 (0.944)	5.550 (0.736)	5.702 (1.226)
Thymus	Rel Body	0.194 (0.030)	0.175 (0.030)	0.175 (0.030)	0.186 (0.030)
	Rel Brain	27.517 (4.855)	23.852 (4.520)	23.837 (5.424)	25.782 (4.760)
Adrenal Glands	Rel Body	0.030 (0.006)	0.030 (0.004)	0.033 (0.006)	0.031 (0.006)
	Rel Brain	4.259 (0.762)	4.152 (0.564)	4.378 (0.569)	4.211 (0.652)
Pituitary	Rel Body	0.006 (0.002)	0.006 (0.002)	0.006 (0.001)	0.005 (0.001)
	Rel Brain	0.852 (0.190)	0.742 (0.209)	0.848 (0.200)	0.720 (0.090)

bw = body weight, Cg = coagulating glands, CX = cervix, fluid = accessory fluids, OD = oviducts, Rel = relative, S.D. = standard deviation, Sem Ves = seminal vesicles

** = Significantly different from the control group at 0.01 using Dunnett's test

Figure 1

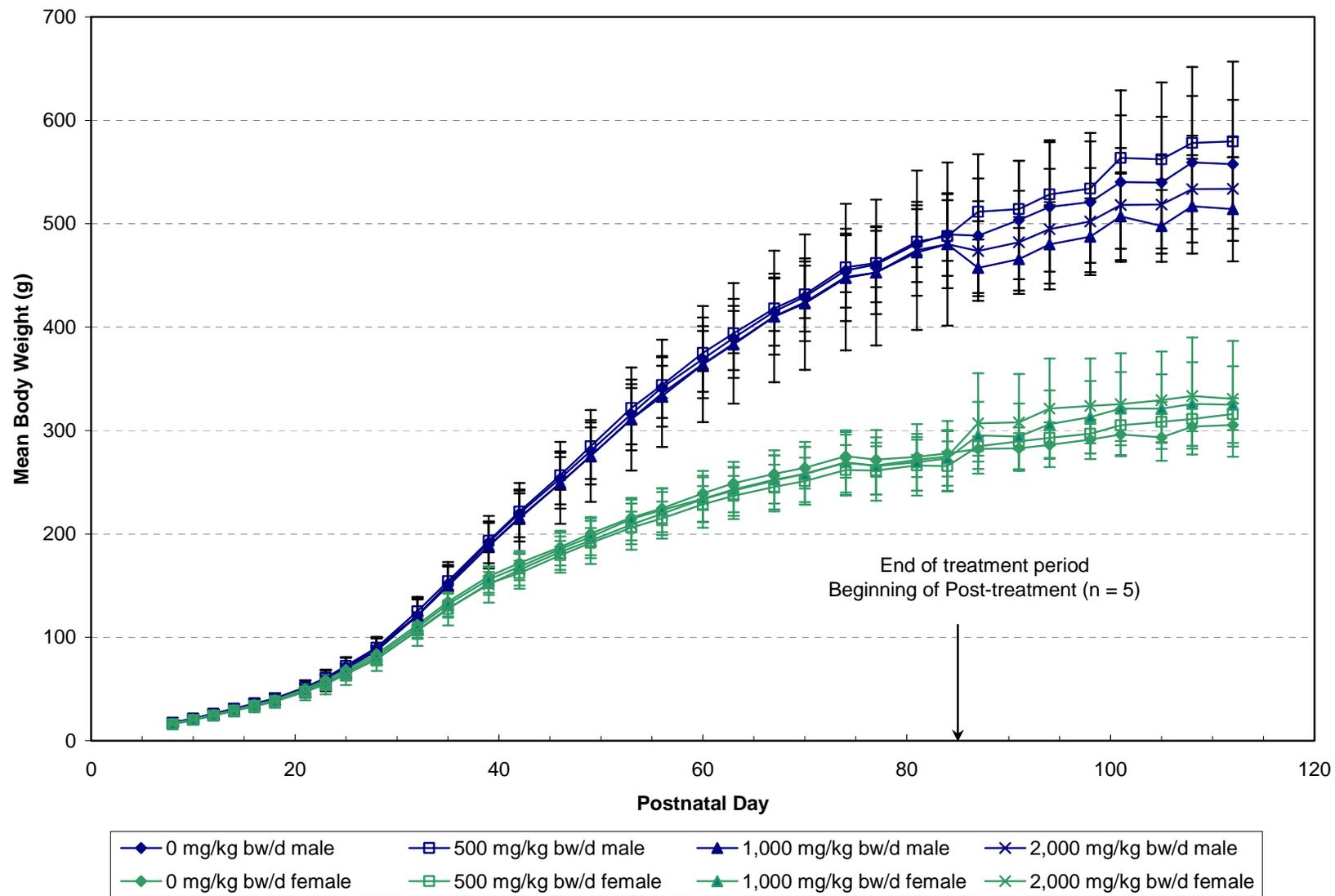


Figure 2

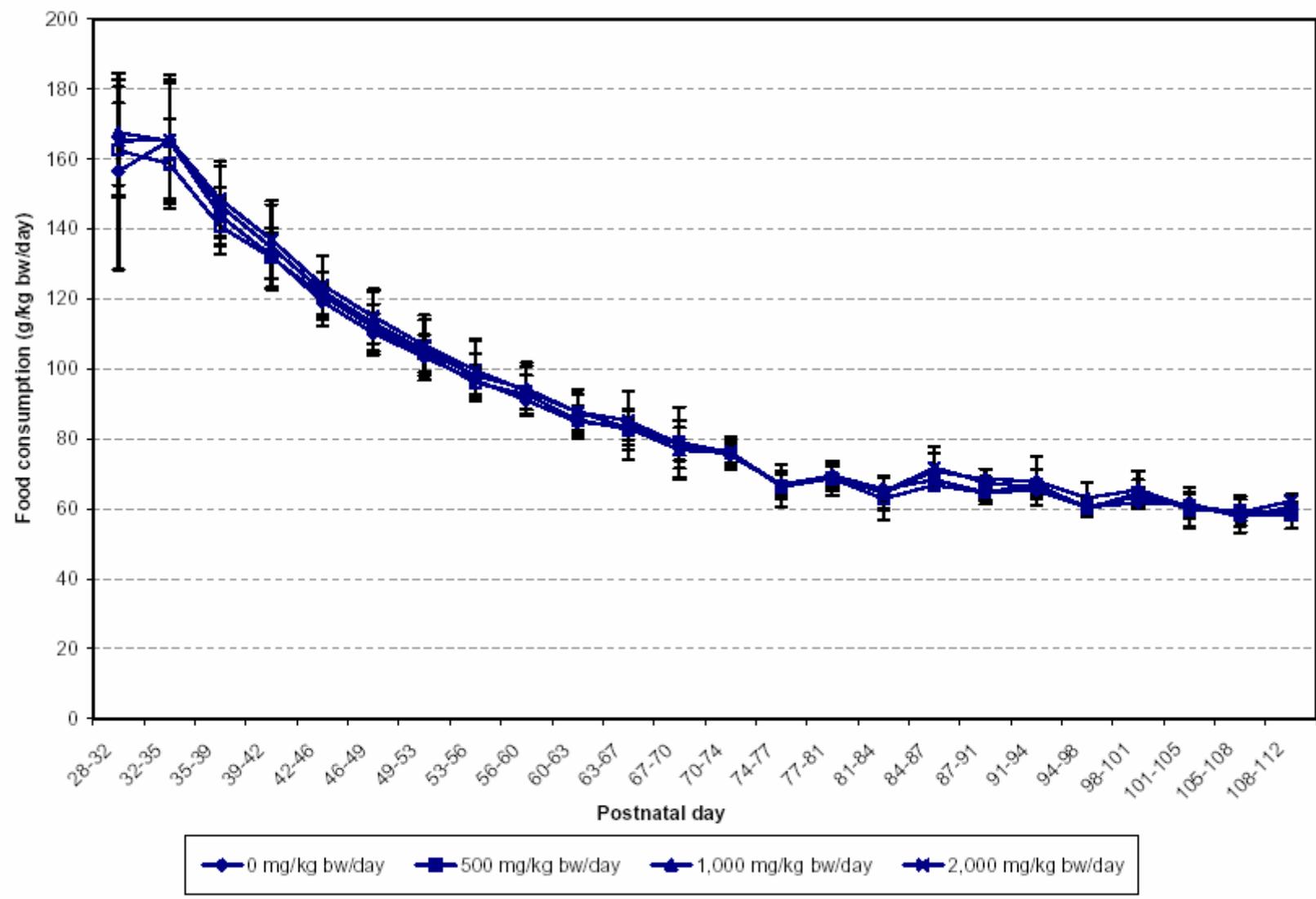
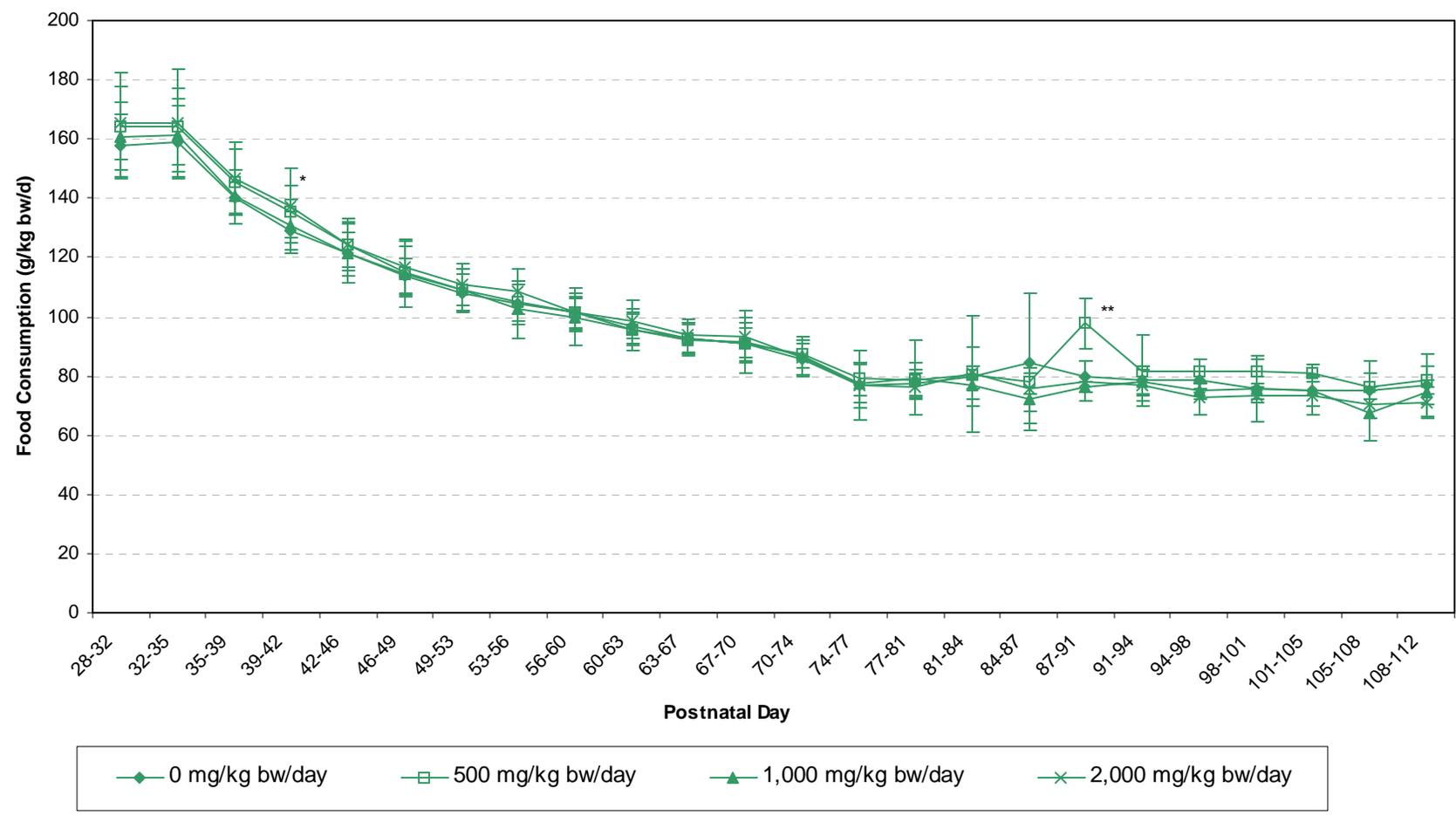


Figure 3



I Additional Details Regarding the Basis for Generally Recognized as Safe (GRAS) Determination of KiwiBerry Concentrate

As noted in the KiwiBerry Concentrate Notification, submitted to the U.S. Food and Drug Administration by Efficas, Inc. and dated October 4, 2006, KiwiBerry Concentrate is produced by concentrating the hot water soluble components of dried hardy kiwi fruit, *Actinidia arguta*, 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 can be dried to produce a tan powdered concentrate (KiwiBerry Powder Concentrate).

Comparative analysis of KiwiBerry Concentrate and hardy kiwi fruit demonstrates that they are very similar in composition, and hence, the essential equivalence of KiwiBerry Concentrate to its parent fruit, *A. arguta*, provides the basis for GRAS using scientific procedures. The essential equivalence of KiwiBerry Concentrate and *A. arguta* fruit to the common green kiwi, *A. deliciosa*, provides additional supportive evidence of safety of the ingredient. Values from the published literature and comparative analyses of the ingredient to samples of the fruits were evaluated. Seven (7) independent lots of KiwiBerry Concentrate, produced from 7 lots of fruit harvested over a 3-year period, were subjected to compositional analysis and were compared to the composition of fresh fruit samples.

Ash, Protein, Fat, and Carbohydrate Content

Levels of the macro components, ash, protein, and total carbohydrate, as well as caloric content of the ingredient, are comparable to the fresh fruit. The values of levels of fat in KiwiBerry Concentrate are reduced approximately 35% compared to values for the fruit, probably due to the processing method but possibly also due in part to seasonal variation. This reduction in fat of approximately 2 g/100 g does not constitute a safety concern. Macro- and micro- constituents of a variety of fruits typically display a range of variation from 1.5- to 18.5-fold, depending upon the constituent (IFBC, 1990).

The sugars present in *A. arguta* are greatly impacted by ripeness (Boyes *et al.*, 1997; Klages *et al.*, 1998; Boldingh *et al.*, 2000). The mean total sugars content in KiwiBerry Concentrate is 19% higher than the upper range of published values for *A. arguta* fruit in New Zealand, but this does not constitute a safety concern. This minor difference may reflect the fact that fruit used for processing tends to be riper than fruit picked for fresh consumption, or may be due to varietal, seasonal, or environmental variation or a combination of these factors. The difference in sucrose:monosaccharide ratio between the ingredient and published values from New Zealand is most likely 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. No attempt is made to inactivate invertase during the manufacturing of KiwiBerry Concentrate, and sucrose levels are very low.

The published sugar:acid ratio in *A. arguta* is 4.33 ± 0.45 (Boyes *et al.*, 1997), and the ratio of mean total sugars to mean total acids in KiwiBerry Concentrate is 4.3, which is entirely equivalent. The amount of total organic acids (sum of citric, malic, and quinic acids) in KiwiBerry Concentrate ranges from 100 to 142.5 mg/g dry weight, which is comparable to average published values for the fruit harvested in Japan [*i.e.*, 117 mg/g dry weight (Okamoto and Goto, 2005)], but somewhat higher than levels reported for the fruit grown in New Zealand [*i.e.*, 80 mg/g dry weight (Boyes *et al.*, 1997)]. The organic acids present in *A. arguta* are impacted by ripeness (Boyes *et al.*, 1997) and probably also by variety and environmental factors. Thus, the organic acid content of KiwiBerry Concentrate is essentially equivalent to that of the fruit.

Mineral Content

Following analysis, the composition of magnesium and phosphorus was identified to be equivalent between KiwiBerry Concentrate and kiwi fruit. Some minor differences in mineral content between the ingredient and published values for fruit from Japan (Okamoto and Goto, 2005), which are deemed of no concern, were noted. The mineral content of fruits typically displays a range of variation from 1.5- to 3-fold (calcium), from 1.4- to 1.7-fold (potassium) and from 2.7- to 10-fold (sodium), depending upon the fruit assayed (IFBC, 1990). Average calcium levels in KiwiBerry Concentrate were 41% lower than the published value, but this is of no consequence because it is not outside of the range of variation expected, and neither the ingredient nor the fruit are a significant source of dietary calcium [$<1\%$ of Daily Reference Value (DRV)]. Average potassium levels in KiwiBerry Concentrate were 53% higher than the published value, but this is of no consequence because it is not outside of the range of variation expected and neither the ingredient nor the fruit are a significant source of dietary potassium ($<3\%$ of DRV). Additionally, average sodium levels in KiwiBerry Concentrate were 6.7-fold higher than in the fruit, but this also is of no consequence because it is not outside of the range of variation expected and neither the ingredient nor the fruit are a significant source of dietary sodium ($<0.1\%$ of DRV). Thus, the mineral content of the ingredient is essentially equivalent to that of the fruit.

Anthocyanin Content

The major anthocyanin in *A. arguta*, cyanidin, was apparently lost during the manufacturing of KiwiBerry 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 Concentrate.

None of the anthocyanins contained in hardy kiwi were observed to be disproportionately concentrated during the KiwiBerry Concentrate manufacturing process.

Flavonoid Content

Flavonoids detected in KiwiBerry Concentrate also were detected in the fresh *A. arguta* fruit and/or in *A. deliciosa*. The levels of isorhamnetin and kaempferol present in KiwiBerry Concentrate produced from fruit harvested during 2004 and 2005 were not detected in the one lot of *A. arguta* analyzed from the 2003 harvest, nor in the lot of KiwiBerry Concentrate produced from fruit harvested in 2003, but are within range of those identified in fresh *A. deliciosa*. Neither of these flavonoid components are essential nutrients, so their seasonal variation is of no consequence.

Quercetin levels in individual lots of KiwiBerry Concentrate ranged from 21.5 to 82.4 ppm, and were 36.07 and 16.71 ppm in the single samples of *A. arguta* and *A. deliciosa* fruit tested, respectively. KiwiBerry Concentrate produced from *A. arguta* fruit harvested in 2003 had lower levels of quercetin (21.5 ppm) than ingredient produced from fruit harvested during 2004 and 2005 (range 48.85 to 82.44 ppm). *A. arguta* fruit from the 2003 harvest also had relatively low levels of quercetin (36.07 ppm). Thus lot to lot differences in quercetin content are most likely due to seasonal variation. None of the flavonoids contained in hardy kiwi were observed to concentrate disproportionately during the KiwiBerry Concentrate manufacturing process. Thus, overall, the flavonoid composition of KiwiBerry Concentrate and the fruit are essentially equivalent.

Data Supporting the Safety of Dietary Quercetin

Dietary record-based cohort assessments conducted in various populations (e.g., Australia, the Netherlands, Finland, Italy, Croatia, Serbia, Greece, Japan, and the U.S.) have indicated mean consumption levels of quercetin from the diet in the range of 2.6 to 38.2 mg/day (mean intakes ranged from 5.4 to 16.8 mg/day in the U.S. study populations) (Hertog *et al.*, 1995; Rimm *et al.*, 1996; Knekt *et al.*, 1997; Kimira *et al.*, 1998; Johannot and Somerset, 2006; Lin *et al.*, 2006); however, high-end consumers of fruits and vegetables, particularly in cases where the individuals consume the peel portion of quercetin-rich fruits and vegetables such as apples, tomatoes, and onions, can consume daily levels of quercetin as high as 200 to 500 mg (Jones and Hughes, 1982; USDA, 2000). In the 7 lots of KiwiBerry Concentrate analyzed, the concentration of quercetin ranged from 21.5 to 82.44 ppm. For the average and 90th percentile user, consuming 2.39 and 4.4 g KiwiBerry Concentrate/day, respectively (all proposed uses), this represents a daily quercetin intake of 51 to 197 µg and 95 to 363 µg, respectively. For comparative purposes, one medium red delicious apple provides approximately 6 mg quercetin (Harnly *et al.*, 2006), a level that is at least 16.5-fold greater than the level

ADDENDUM - KIWIBERRY CONCENTRATE NOTIFICATION

of daily quercetin exposure estimated for a 90th percentile consumer of KiwiBerry Concentrate under the intended conditions of use as an ingredient in foods. Furthermore, the 90th percentile intake of quercetin from the intended conditions of use of KiwiBerry Concentrate (95 to 363 µg/day) is much lower than the reported U.S. dietary intake of quercetin (*i.e.*, 5.4 to 16.8 mg/day).

A considerable amount of information has been reported in the scientific literature with respect to quercetin safety. The majority of results from numerous *in vitro* assays of quercetin-related mutagenicity and genotoxicity have been positive; however, these findings have not been confirmed by *in vivo* experiments. With oral administration to mice and rats, quercetin consistently did not induce any significant changes in several mutagenicity/genotoxicity endpoints (*i.e.*, micronuclei, chromosomal aberrations, sister chromatid exchange, unscheduled DNA synthesis, and alkali-labile DNA damage) in somatic cells in comparison to untreated controls. The absence of *in vivo* mutagenic and genotoxic effects may be attributable to the limited absorption, extensive intestinal degradation, and post-absorption enzymatic metabolism following quercetin consumption.

The potential carcinogenicity due to oral administration of quercetin has been evaluated in many long-term animal studies, of which a majority have indicated no evidence of significantly increased incidences of tumor formation (Ambrose *et al.*, 1952; Saito *et al.*, 1980; Hirono *et al.*, 1981; Hosaka and Hirono, 1981; Morino *et al.*, 1982; Takanashi *et al.*, 1983; Stoewsand *et al.*, 1984; Ito *et al.*, 1989); however, equivocal results for the potential quercetin-related carcinogenicity in rats provided high amounts of quercetin in the diet were indicated in 2 studies (Pamukçu *et al.*, 1980; Dunnick and Hailey, 1992; NTP, 1992). In a 2-year carcinogenicity bioassay conducted by the National Toxicology Program (NTP), an increased severity of chronic nephropathy, hyperplasia, and neoplasia of the renal tubular epithelium, resulting primarily in benign tumors of the renal tubular epithelium, was reported in male but not female F344/N rats exposed to quercetin at a dietary level of 40,000 ppm (approximately 2,000 mg/kg body weight/day) (Dunnick and Hailey, 1992; NTP, 1992). At lower dietary levels of 1,000 and 10,000 ppm quercetin (providing approximately 50 and 500 mg/kg body weight/day, respectively), no statistically significant adverse effects were reported. The authors concluded that the results of this study provided some evidence of carcinogenic activity of quercetin in male F344/N rats due to an increased incidence of renal tubule cell adenomas; however, in a re-evaluation of this NTP study, Hard *et al.* (2007) suggested that the renal lesions observed were mediated by the exacerbation of chronic nephropathy which is a spontaneous, age-related, rodent-specific disease. Late-stage chronic progressive nephropathy in the male rat has been associated with increased background incidences of tumor types and may be potentiated by exogenous compounds. In the re-evaluation, the majority of the tumors identified (*i.e.*, small-sized

adenomas, borderline with atypical tubule hyperplasia) were consistent with the criteria for the development of kidney tumors as a result of chemically-exacerbated chronic nephropathy response. Also, cellular alterations indicative of chemically-induced toxicity were not observed. Hard *et al.* (2007) concluded that the kidney lesions observed in the NTP study were associated with late-stage chronic progressive nephropathy in the male rat, which was further substantiated by the absence of nephrotoxicity in female rats studied.

Pamukçu *et al.* (1980) demonstrated an increased incidence of urinary bladder and intestinal tumors in Norwegian rats provided a comparatively lower dietary dose of quercetin (approximately 50 mg/kg body weight/day) for a period of 58 weeks; however, these results were not confirmed in longer-term studies performed using doses of quercetin several-fold higher, including the 2-year NTP study (Saito *et al.*, 1980; Hirono *et al.*, 1981; Hosaka and Hirono, 1981; Takanashi *et al.*, 1983; Ito *et al.*, 1989; Dunnick and Hailey, 1992; NTP, 1992). Several reasons to account for the inconsistency between the findings of Pamukçu *et al.* (1980) and the other long-term studies, including strain- or species-specific differences in tumor susceptibility, variations in experimental conditions, differences in basal diets, and potential cross-contamination of the diet with bracken fern, a known inducer of urinary bladder and intestinal tumors, are possible and have been suggested by other authors (Wang *et al.*, 1976; Saito *et al.*, 1980; Hirono *et al.*, 1981; Hosaka and Hirono, 1981; Morino *et al.*, 1982; Takanashi *et al.*, 1983; Lamson and Brignall, 2000; Okamoto, 2005), and in the absence of replicate findings in numerous subsequent carcinogenicity studies, the relevance of the results of Pamukçu *et al.* (1980) in an assessment of the safety of dietary quercetin cannot be afforded credence.

In clinical studies in which quercetin or plant extracts containing quercetin glycosides were provided to study subjects as oral preparations for consumption for periods of up to 12 weeks at dose levels ranging between 3 and 1,000 mg quercetin/day, no compound-related adverse effects were reported (Shoskes *et al.*, 1999; Kiesewetter *et al.*, 2000; Lozoya *et al.*, 2002). No adverse effects were reported in male and female subjects following ingestion of single oral doses of quercetin (8.5 mg/kg body weight/day or approximately 500 mg for an average 60 kg individual) in a study demonstrating a quercetin-related increase in adenosine levels (Blardi *et al.*, 1999). Moreover, quercetin has been marketed in the U.S. as a dietary supplement for at least prior to October 15, 1994 (UNPA, 1999), and likely longer, with recommended daily doses of supplemental quercetin in the range of 200 to 1,200 mg (PDRNS, 2001), without evidence of quercetin-related toxicity under these recommended conditions of use.

In summary, on a body weight basis, the 90th percentile estimated intake level of quercetin from the proposed use of KiwiBerry Concentrate (95 to 363 µg/day) is several

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orders of magnitude less than the quercetin dose level of 500 mg/kg body weight/day used in the NTP 2-year rat study, at which level no toxicologically significant adverse effects were observed (Dunnick and Hailey, 1992; NTP, 1992; Hard *et al.*, 2007). In clinical trials, doses of quercetin up to 1 g/day have been utilized without adverse effects. In addition, daily doses of 200 to 1,200 mg/day of supplemental quercetin have been recommended for use for several years, which are levels that are at least 550-fold greater than the estimated daily exposure of 363 µg quercetin that would occur under the intended conditions of use of KiwiBerry Concentrate in foods.

Conclusion

In conclusion, the composition of KiwiBerry Concentrate is essentially equivalent to the parent fruit, *A. arguta*, and there are no concerns for potential increases in exposure of any of the constituents of the fruit as a result of the concentration process used to manufacture the ingredient, nor are there any anticipated adverse effects on human health of any of the components of KiwiBerry Concentrate. Therefore, KiwiBerry Concentrate, meeting appropriate food-grade specifications and manufactured in accordance with cGMP, is GRAS for the conditions of intended use described in the original Notification, dated October 4, 2006, based on scientific procedures.

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