#873

Ocean Spray Cranberries, Inc.
One Ocean Spray Drive, Lakeville-Middleboro, MA 02349
p (508) 946-1000, f (508) 946-7704

26 June 2019

Office of Food Additive Safety HFS-200
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Drive
College Park, MD, 20740

Dear Sir or Madam:

Accompanying this letter is a notice pursuant to regulations of the Food and Drug Administration found at 21 CFR Part 170 setting forth the basis for the conclusion reached by the submitter, Ocean Spray Cranberries, Inc, that cranberry extract powder, is generally recognized as safe under the intended conditions of use described in the notice. The notice is contained in a binder. In addition, we include a CD that contains a complete copy of the notice. I hereby certify that the electronic files contained on the CD were scanned for viruses prior to submission, and thus certified as being virus-free using Symantec Endpoint Protection.

Sincerely,

[redacted]

Christina Khoo
Director, Global Health Science and Nutrition Policy
Dept Health Science and Nutrition Affairs
Ocean Spray Cranberries, Inc
Lakeville MA 02347
ckhoo@oceanspray.com

GRAS NOTICE FOR CRANBERRY EXTRACT POWDER

SUBMITTED TO:
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration
5001 Campus Drive
College Park, MD
20740 USA

SUBMITTED BY:
Ocean Spray Cranberries, Inc.
One Ocean Spray Drive
Lakeville-Middleboro, MA
02349 USA

DATE:
07 June 2019

GRAS Notice for Cranberry Extract Powder

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GRAS Notice for Cranberry Extract Powder

Part 1. §170.225 Signed Statements and Certification

In accordance with 21 CFR §170 Subpart E consisting of §170.203 through 170.285, Ocean Spray Cranberries, Inc. (hereafter Ocean Spray) hereby informs the United States (U.S.) Food and Drug Administration (FDA) that the intended uses of cranberry extract powder, as manufactured by Ocean Spray, in various conventional food and beverage products as described in Section 1.3 below, are not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on Ocean Spray's view that these notified uses of cranberry extract powder are Generally Recognized as Safe (GRAS). In addition, as a responsible official of Ocean Spray, the undersigned hereby certifies that all data and information presented in this notice represents a complete and balanced submission that is representative of the generally available literature. Ocean Spray considered all unfavorable as well as favorable information that is publicly available and/or known to Ocean Spray and that is pertinent to the evaluation of the safety and GRAS status of cranberry extract powder as a food ingredient for addition to cranberry extract powder food products, as described herein.

Signed,

[redacted]

Digitally signed by Christina Khoo
DN: cn=Christina Khoo, o=Ocean Spray Cranberries, Inc., ou, email=ckhoo@oceanspray.com, c=US
Date: 2019.06.25 13:33:38 -04'00'

6/25/19

Christina Khoo
Director, Global Health Science and Nutrition Policy
Dept Health Science and Nutrition Affairs

Date

1.1 Name and Address of Notifier

Ocean Spray Cranberries, Inc.
One Ocean Spray Drive
Lakeville-Middleboro, MA
02349 USA

1.2 Common Name of Notified Substance

Cranberry extract powder

1.3 Conditions of Use

Ocean Spray intends to market cranberry extract powder in beverages and beverage bases and processed fruits and fruit juices. The main components in cranberry extract powder include polyphenols, specifically proanthocyanidins (~55%), anthocyanins (5 to 8%), phenolics (35 to 38%), and organic acids (0.4 to 0.5%).

A summary of the food categories and use-levels in which cranberry extract powder is intended for use is provided in Table 1.3-1 below. Food-uses are organized according to 21 CFR §170.3 (U.S. FDA, 2018).

The use-level of cranberry extract powder for beverages and beverage bases, and coffee and tea are 150 mg/8 oz serving or 62.5 mg/100 g. This is equivalent to:

- 33.3 mg/100 g of proanthocyanidins;
- 5.0 mg/100 g of anthocyanins;
- 25.4 mg/100 g of phenolics; and
- 0.4 mg/100 g of organic acids.

The use-level of cranberry extract powder for processed fruits and fruit juices is 300 mg/8 oz serving or 125 mg/100 g. This is equivalent to:

- 66.6 mg/100 g of proanthocyanidins;
- 10.0 mg/100 g of anthocyanins;
- 50.8 mg/100 g of phenolics; and
- 0.8 mg/100 g of organic acids.

Table 1.3-1 Summary of the Individual Proposed Food-Uses and Use-Levels for Cranberry Extract Powder in the U.S.

Food Category (21 CFR §170.3) (U.S. FDA, 2018)	Food-Uses	RACC[a] (mL)	Cranberry Extract Powder Level (mg/8 oz serving)	Cranberry Extract Powder Use-Levels (mg/100 g)
Beverages and Beverage Bases	Energy Drinks	360	150	62.5
	Enhanced or Fortified Waters	360	150	62.5
	Flavored or Carbonated Waters	360	150	62.5
	Sport and Electrolyte Drinks, Fluid Replacement Drinks	360	150	62.5
Coffee and Tea	Ready-to-Drink Cold brew Coffee Drinks	360	150	62.5
Processed Fruits and Fruit Juices	Fruit Drinks and Ades (Ready-to-Drink and Powder Mixes)	240	300	125

CFR = Code of Federal Regulations; RACC = Reference Amounts Customarily Consumed per Eating Occasion; U.S. = United States.
[a] RACC based on values established in 21 CFR §101.12 (U.S. FDA, 2018). RACCs are included for reference; however, the assessment was conducted based on use-levels expressed per 100 g.

Table 1.3-2 provides a comparison of the proposed use-levels of proanthocyanidins, anthocyanidins, and phenolics from the proposed conditions of use for cranberry extract powder to those found in other cranberry beverages. The proposed use of cranberry extract results in concentrations of these components comparable to what is found in 27% cranberry juice cocktail, but less than those found in 100% cranberry juice.

Table 1.3-2 Comparison of Phenolic Levels Under Proposed Conditions of Use to Those in Cranberry Juice

Constituents (mg/8 oz serving)	Cranberry Beverage Products			Proposed Use of Cranberry Extract Powder	
	100% Cranberry Juice in Beverages (Unsweetened)	27% Cranberry Juice[a]	Cranberry Juice Cocktail	150 mg/8 oz serving	300 mg/8 oz serving
Proanthocyanidins	576[b] (481–916)	156	90 (70–140)	82.5	165
Phenolics	736	199	211	7.5	15
Anthocyanins	53	14	12	52.5	105

[a] These values are calculated on the basis of the provided figures for pure (100%) cranberry juice and does not consider any addition other than water, noting that existing 27% cranberry juices on the market may contain higher amounts of some of these constituents deriving from other fruit/vegetable juice used for blending.
[b] Mean and range on the basis of 64 samples over several years.

1.4 Basis for GRAS

Pursuant to 21 CFR §170.30 (a)(b) of the Code of Federal Regulations (CFR) (U.S. FDA, 2018), Ocean Spray has concluded that the intended uses of cranberry extract powder as described herein are GRAS on the basis of scientific procedures.

1.5 Availability of Information

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

Ocean Spray Cranberries, Inc.
One Ocean Spray Drive
Lakeville-Middleboro, MA
02349 USA

Should the FDA have any questions or additional information requests regarding this Notification, Ocean Spray will supply these data and information upon request.

1.6 Freedom of Information Act, 5 U.S.C. 552

It is Ocean Spray's view that all data and information presented in Parts 2 through 7 of this Notice do not contain any trade secret, commercial, or financial information that is privileged or confidential, and therefore, all data and information presented herein are not exempted from the Freedom of Information Act, 5 U.S.C. 552.

Part 2. §170.230 Identity, Method of Manufacture, Specifications, and Physical or Technical Effect

2.1 Identity

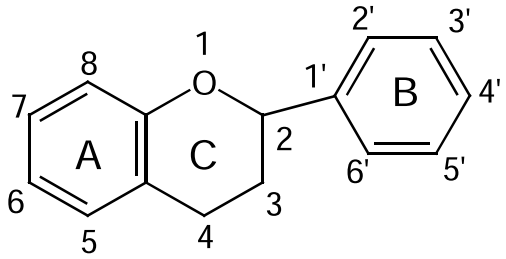
Cranberry extract powder is a water soluble phenolic-rich powder extract of cranberry *(Vaccinium macrocarpon)* juice. The current taxonomic placement of *Vaccinium macrocarpon* is summarized below:

Kingdom: *Plantae*
Phylum: *Magnoliophyta*
Class: *Magnoliopsida*
Order: *Ericales*
Family: *Ericaceae*
Genus: *Vaccinium*
Species: *Vaccinium macrocarpon*

The powder extract has a dark purple color, with little to no odor, and is bitter and astringent in taste.

The phenolic fraction of cranberry juice is primarily composed of what has been termed polyphenols that consist of a number of different flavonoid structures in addition to more simple phenolic acids like hydroxybenzoic acids. The general structure for these types of substances is described in Figure 2.1-1.

Figure 2.1-1 The Generic Structure of Flavonoids

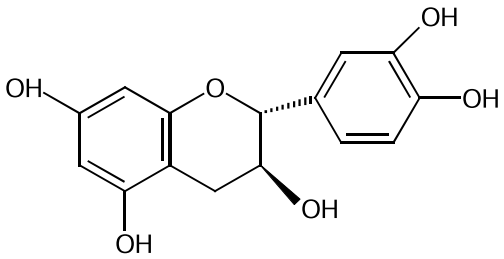


The flavonoids are divided into 6 subtypes depending on the oxidation state of the central pyran ring, 3 of which are represented in the phenolic fraction from cranberry juice including flavanols [proanthocyanidins (oligomers and polymers of catechins), the anthocyanins that contain a C3 hydroxyl group and unsaturated C ring], and flavonols that contain a C3 hydroxy group, a C4 keto substituent, and unsaturated C ring. Each of these groups of compounds is discussed separately below.

2.1.1 Proanthocyanidins

Proanthocyanidins are a class of nutrients that belong to the flavonoid family and can be described as a mixture of procyanidin oligomers and polymers. Examples of oligomers and polymers of procyanidins are shown below in Figures 2.1.1-1, 2.1.1-2, and 2.1.1-3.

Figure 2.1.1-1 Structure of (+)Catechin Monomer of Procyanidins



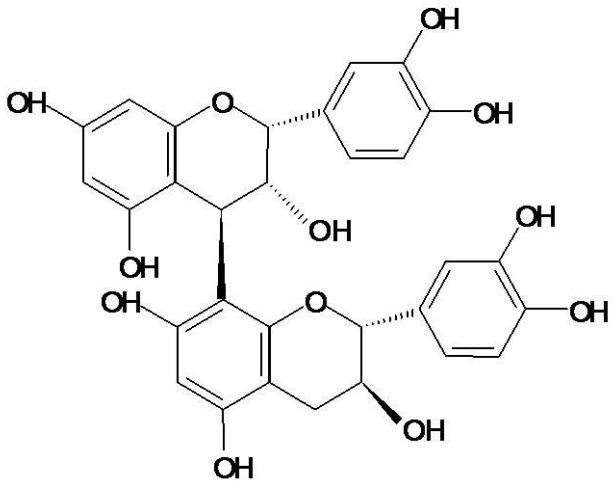
Synonyms: (2R-*trans*)-2-(3,4-dihydroxyphenyl)-3-4-dihydro-2H-1-benzopyran-3,5,7-triol; catechol; catechin; 3,3',4',5,7-flavanpentol; catechinic acid; catechuic acid; cianidanol; dexcyanidanolcyanidol; (+)-cyanidanol-3; Catergen

CAS Number: 154-23-4

Empirical Formula: $C_{15}H_{14}O_6$

Molecular Weight: 290.272

Figure 2.1.1-2 Structure of Proanthocyanidin B-1 Dimer



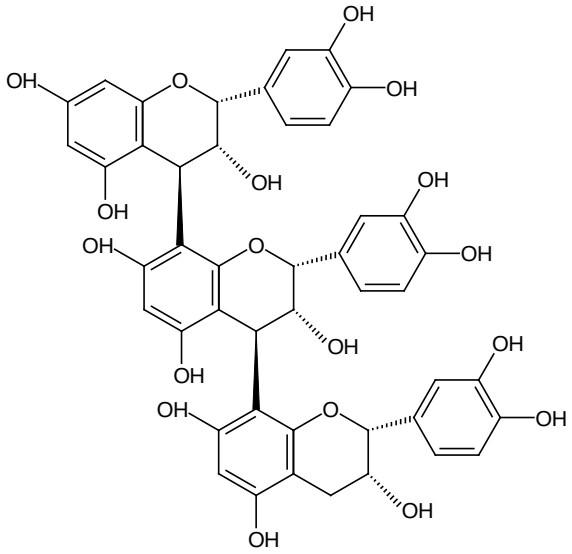
Synonyms: Procyanidin B1; Cis,Trans''-4,8''-BI-(3,3',4',5,7-Pentahydroxyflavane); Epicatechin(4B-8)Catechin; Epicatechin(4beta->8)Catechin; (2R,2'R,3R,3'S,4R)-2,2'-Bis(3,4-dihydroxyphenyl)-4,8'-bichroman-3,3',5,5',7,7'-hexol; (2R,2'R,3R,3'S,4β)-3,3',4,4'-Tetrahydro-2α,2'α-bis(3,4-dihydroxyphenyl)-4,8'-bi[2H-1-benzopyran]-3,3',5,5',7,7'-hexol; (2R,3R,4R,2'R,3'S)-2,2'-Bis(3,4-dihydroxyphenyl)-3,3',4,4'-tetrahydro-4,8'-bi(2H-1-benzopyran)-3,3',5,5',7,7'-hexol

CAS Number: 20315-25-7

Empirical Formula: $C_{30}H_{26}O_{12}$

Molecular Weight: 578.52

Figure 2.1.1-3 Structure of Proanthocyanidin C-1 Trimer

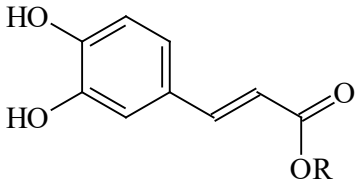


Synonyms: Epicatechin-(4b,8)-epicatechin-(4b,8)-epicatechin
CAS Number: n/a
Empirical Formula: $C_{45}H_{38}O_{18}$
Molecular Weight: 866

2.1.2 Phenolic Acids

Caffeic acid, chlorogenic acid, and hydroxycinnamic acid represent some of the phenolic acids present in cranberry extract powder. The general structure for caffeic and chlorogenic acid is presented in Figure 2.1.2-1. In the structure below, caffeic acid would occur when the R was a hydrogen molecule. For chlorogenic acid the R would represent a glucose molecule.

Figure 2.1.2-1 General Structure for Caffeic Acid/Chlorogenic Acid

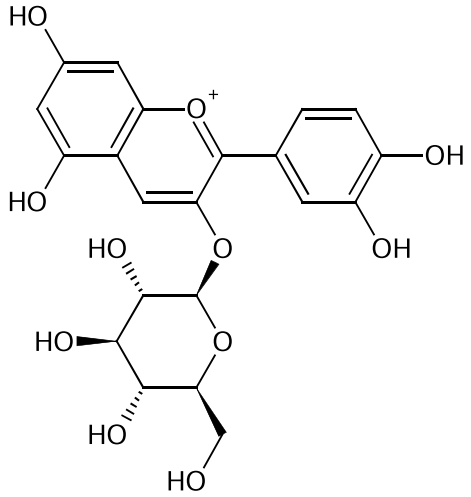


Caffeic acid		**Chlorogenic acid**	
Synonyms:	3,4-dihydroxycinnamic acid	Synonyms:	3-caffeoylquinic acid, 3-(3,4-dihydroxycinnamoyl)quinic acid
CAS Number:	331-39-5	CAS Number:	327-97-9
Empirical Formula:	$C_9H_8O_4$	Empirical Formula:	$C_{16}H_{18}O_9$
Molecular Weight:	180.16	Molecular Weight:	354.31

2.1.3 Anthocyanins

Anthocyanins are pigment molecules that occur in a wide variety of plants including cranberries. In general, anthocyanins contain a positively charged oxygen atom, known as a benzopyrilium cation, which is usually associated with a chloride anion. Cranberry extract powder contains different glycosides of the basic 3-glucoside presented below in Figure 2.1.3-1. These include the galactoside and arabinoside forms in addition to the same glycosides of peonidin where one of the ring B phenolic groups contains a methoxy substituent.

Figure 2.1.3-1 Structure of Cyanidin-3-Glucoside

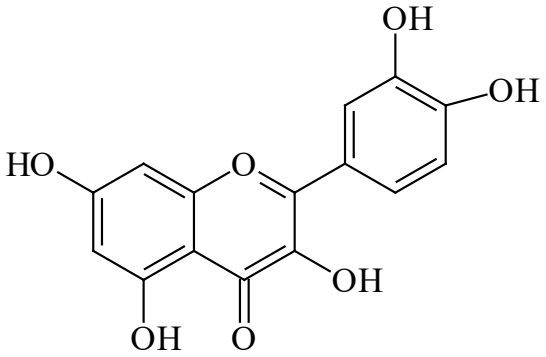


Synonyms: Asterin, chrysontemin, cyanidin 3-O-glucoside, cyanidin 3-monoglucoside, cyanidine 3-glucoside, cyanidol 3-glucoside, glucocyanidin
CAS Number: 7084-24-4
Empirical Formula: $C_{21}H_{21}O_{11}$
Molecular Weight: 484.84

2.1.4 Flavonols

A number of different flavonols are present in the cranberry extract powder including quercetin, quercitrin, hyperoside, and myricetin. All of these display a structure that is similar to that of quercetin. As compared to quercetin, quercitrin has a 3-rhamnose substituent while hyperoside has a 3-galactose substituent. Myricetin has an additional hydroxyl on the C ring phenol group. The structure of quercetin is presented below in Figure 2.1.4-1 as an example of the flavonols present in cranberry extract powder.

Figure 2.1.4-1 Structure of Quercetin



Synonyms: Quercitin, 3,3',4',5,7-pentahydroxyflavone
CAS Number: 117-39-5
Empirical Formula: $C_{15}H_{10}O_7$
Molecular Weight: 302.24

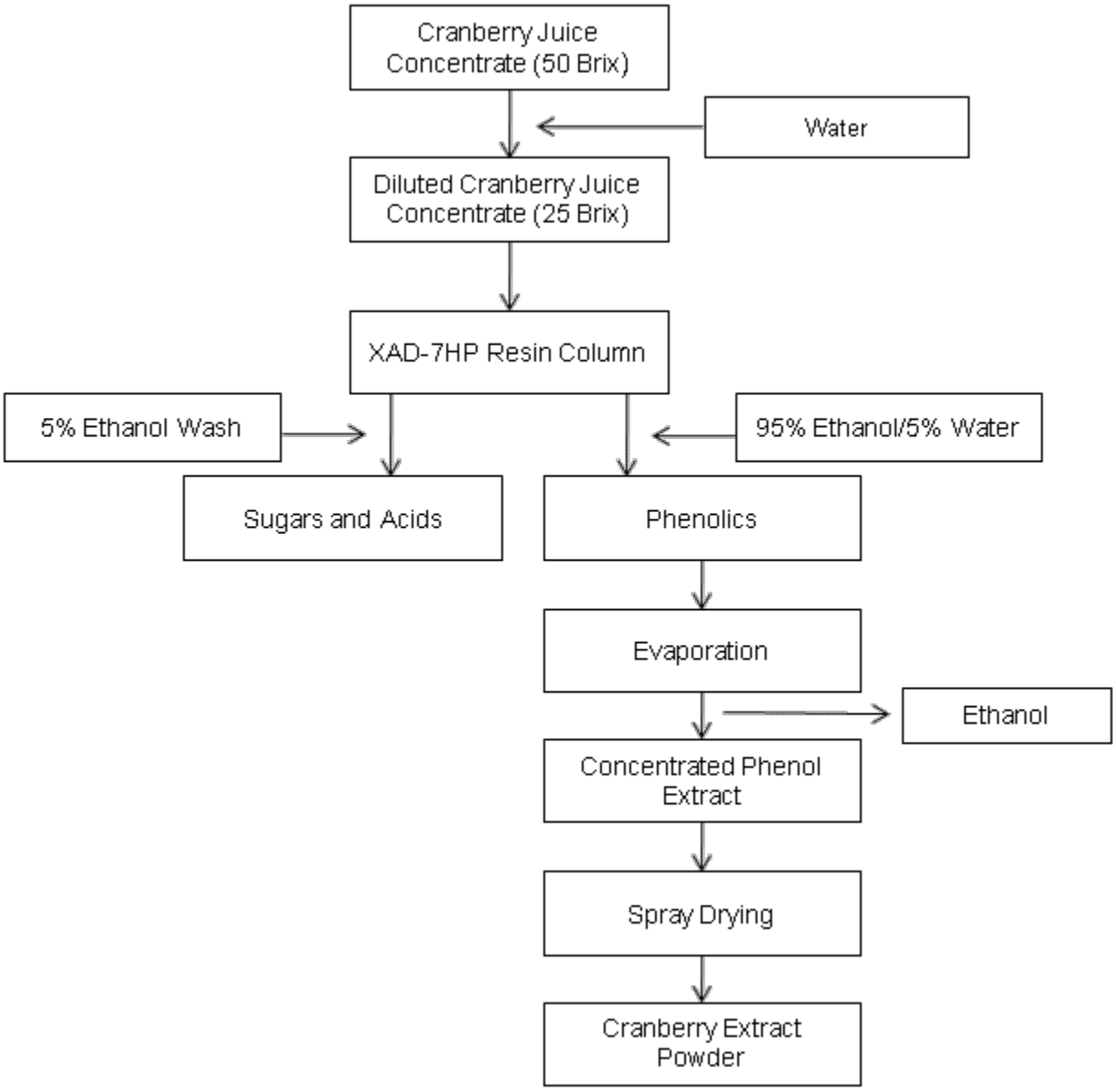
2.2 Manufacturing

2.2.1 Overview

Typically, cranberry juice contains sugars, organic acids, and phenolic compounds (including flavonols, flavan-3-ols, anthocyanins, and proanthocyanidins). Of the solid material in cranberry juice, 89.8% (±2.0%) are organic acids and sugars (Ocean Spray's internal compositional cranberry juice database). These phenolic compounds are selectively isolated from the other components of cranberry juice to yield cranberry extract.

Initially, food-grade cranberry juice concentrate is diluted from a product with a percentage soluble solids or standard degrees Brix of 50 to 25 degrees Brix. The diluted concentrate is then loaded onto a column containing Amberlite™ XAD-7HP absorbent resin (in the process step referred to as loading). After loading, a 5% ethanol wash is used to remove the sugars and organic acids from the resin column. The phenolic components are then eluted from the resin column using a 95% ethanol/5% water solvent mixture. The phenolic fraction is partially concentrated to 25 to 30% solids and the ethanol is recovered, before the concentrated extract is spray dried to yield a powder. Silicon dioxide is added to the powder as a flow agent (0.6 to 0.8% of final product) and maltodextrin is added as a carrier (9 to 22% of final product). The manufacturing process is performed according to current Good Manufacturing Practice (cGMP). A flowchart illustrating the manufacturing steps of cranberry extract powder is detailed in Figure 2.2.1-1.

Figure 2.2.1-1 Schematic Overview of the Manufacturing Process for Cranberry Extract Powder



2.2.2 Raw Materials

The following raw materials are used in the production of cranberry extract powder.

2.2.2.1 Cranberry Juice

Red cranberry juice and concentrate are viscous red liquids prepared from the extracted, depectinized and filtered juice of sound, clean cranberries (*Vaccinium macrocarpon)*. The specifications for the food-grade cranberry juice concentrate employed in the production of cranberry extract powder are provided in Table 2.2.2.1-1.

Table 2.2.2.1-1 Specifications for Cranberry Juice Concentrate

Specification Parameter	Specification	Method of Analysis
Color	Typical dark, clear cranberry red	Visual & QOG Vol. 5, Color Measurement
Haze	<10.0 NTU	QOG Vol. 5, Haze Measurement
Titratable Acidity (w/v as citric)	1.86–2.35%; otherwise blend	QOG Vol. 5, Titratable Acidity Measurement
Pectin	Negative	Alcohol Test
Extraneous Matter	None	Visual
Microbials (Cranberry Juice)		
Yeast	<20,000 CFU/mL	Microbiological methods as described by the Association of Official Analytical Chemists (AOAC), American Public Health Association (APHA) or BAM (Bacteriological Analytical Manual, FDA).
Mold	<1,000 CFU/mL	
Microbials (Cranberry Concentrate)		
Yeast	<3,000 CFU/mL	Microbiological methods as described by the Association of Official Analytical Chemists (AOAC), American Public Health Association (APHA) or BAM (Bacteriological Analytical Manual, FDA).
Mold	<500 CFU/mL	

CFU = colony forming units; FDA = United States Food and Drug Administration; NTU = Nephelometic Turbidity Units.

2.2.2.2 Ethanol

Ethanol, which meets the current specifications laid down by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) is used as an extraction solvent (JECFA, 2006).

2.2.2.3 Absorption Resin

Absorption resins are well-established for food processing. XAD7 HP resin composed of phenol formaldehyde resin is washed according to the instructions of the manufacturer prior to use in food processing.

2.2.3 Additives

Maltodextrin and silicon dioxide are added to the final cranberry extract formulation for technological purposes.

2.2.3.1 Maltodextrin

Maltodextrin is food-grade and also meets the specifications outlined in the European Pharmacopoeia (Ph. Eur., 2004).

2.2.3.2 Silicon Dioxide

Silicon dioxide is used as a flow agent in cranberry extract powder and is typically between 6 and 8 g/kg. In accordance with 21 CFR §172.480 (U.S. FDA, 2018), silicon dioxide is permitted for direct addition to food in an amount not to exceed 2% by weight of the food.

2.3 Product Specifications and Batch Analyses

2.3.1 Chemical Specifications

The proposed chemical specifications for cranberry extract powder are presented in Table 2.3.1-1. Microbial specifications are shown in Table 2.3.1-2.

Table 2.3.1-1 Chemical Specifications for Cranberry Extract Powder

Specification Parameter	Specification	Method of Analysis
Moisture (%)	≤ 5	Gravimetric, Loss of Drying (CEM Microwave Oven, or equivalent)
Proanthocyanidins (%)	≥ 15.0	OSC BL DMAC
Proanthocyanidins (%)	55.0–60.0	OSC-DMAC
Total Phenolics (GAE; %)	> 46.2	Folin

GAE = gallic acid equivalent; HPLC = high-performance liquid chromatography; IR = infrared.

Table 2.3.1-2 Microbial Specifications for Cranberry Extract Powder

Specification Parameter	Specification	Method of Analysis
Yeast and Mold (CFU/g)	<100	Microbiological methods as described by the Association of Official Analytical Chemists (AOAC), American Public Health Association (APHA) or BAM (Bacteriological Analytical Manual, FDA).
Aerobic plate count (CFU/g)	<1,000	
Coliforms (MPN/g)	<3	
Escherichia coli (MPN/g)	<3	
Salmonella (/375 g)	Absent	

CFU = colony forming units; FDA = United States Food and Drug Administration; MPN = most probable number.

2.3.2 Batch Analysis

Batch analysis results for 3 lots of cranberry extract powder representative of the commercial material are presented in Table 2.3.2-1. Certificates of analysis and analytical methodology are provided in Appendices A and B, respectively. Cranberry extract powder is comprised of mainly proanthocyanidins, phenolics, and moisture. Microbial analysis of 3 lots is presented in Table 2.3.2-2. Together, these results indicate that cranberry extract powder can be produced in a reproducible manner consistent with the proposed product specifications.

Table 2.3.2-1 Chemical Analysis for Cranberry Extract Powder

Specification Parameter	Specification	Production Scale Lot		
		8498-09-02B	**8498-09-03**	**8498-09-04**
Moisture (%)	≤ 5	3.53%	4.15%	3.87%
Proanthocyanidins (%) [a]	55.0–60.0	56.80% dwb	58.15% dwb	55.45% dwb
Total Phenolics GAE; %)	> 46.2	49.27% dwb	46.14% dwb	44.91% dwb
		02192016	**02202016**	**05202016**
Proanthocyanidins (%)[b]	≥ 15.0	19.25% dwb	17.90% dwb	18.66% dwb

dwb = dry-weight basis GAE = gallic acid equivalent.
[a] Refer to Appendix B for additional details on the methodology used to quantify the amount of proanthocyanidins and a comparison of both methods (OSC-DMAC and BL-DMAC). For the remainder of the report, the quantity of proanthocyanidins is based on the OSC-DMAC methodology.
[b] Different lots presented for BL-DMAC method as it was developed after analysis of 3 original lots.

Table 2.3.2-2 Microbial Analysis for Cranberry Extract Powder

Specification Parameter	Specification	Production Scale Lot		
		8498-09-02	**8498-09-03**	**8498-09-04-02SD**
Yeast and Mold (CFU/g)	<100	<10	<10	<10
Aerobic plate count (CFU/g)	<1,000	<10	<10	<10
Coliforms (MPN/g)	<3	na	na	<3
Escherichia coli (MPN/g)	<3	na	na	<3
Salmonella (/375 g)	Absent	na	na	Absent

CFU = colony forming units; MPN = most probable number; na = not available.

2.3.3 Product Characteristics

Product characteristics of cranberry extract powder from 3 batch analysis results are illustrated in Table 2.3.3-1.

Table 2.3.3-1 Product Characteristics for Cranberry Extract Powder

Specification Parameter	Production Scale Lot			Reference/Test Methodology
	8498-09-02	**8498-09-03**	**8498-09-04-02SD**	
Appearance	Fine, free-flowing and light-dark red in color	Fine, free-flowing and light-dark red in color	Fine, free-flowing and deep red in color	Sensory.
Screen Analysis	100% through 30 mesh screen	100% through 30 mesh screen	100% through 30 mesh screen	Rotap with hammer, 5 minutes.
Solubility	100%, with no visible insoluble particles	100%, with no visible insoluble particles	100%, with no visible insoluble particles	1.5 g powder in 1,000 mL 50°F water. Powder will dissolve completely in 2 minutes.

2.3.4 Compositional Information

Cranberry extract powder is manufactured from cranberry concentrate *via* a series of physical separation and purification stages. The levels of potentially toxic external contaminants will therefore be controlled by ensuring the quality of the starting cranberry concentrate. Analysis for heavy metals, residual solvents, and microorganisms are shown in Table 2.3.4-1. The results of this analysis demonstrate that heavy metals are present at acceptably low levels. Similarly, the levels of residual ethanol observed from the 3 batches measured of cranberry extract consistently fall below the limit of detection.

Table 2.3.4-1 Product Characteristics for Cranberry Extract Powder

Heavy Metals					
Arsenic (mg/kg)	EPA 200.8	< 3	0.0755	0.0950	0.0719
Cadmium (mg/kg)	EPA 200.8	< 2	<0.01	<0.01	<0.01
Lead (mg/kg)	EPA 200.8	< 10	0.1090	0.1100	0.0423
Mercury (mg/kg)	EPA 200.8	< 1	<0.01	<0.01	<0.01
Residual Solvents					
Ethanol (mg/kg)	AOCS Official Method Ca 3b-87, Hexane Residues in Fats and Oils, or equivalent.	≤ 100	< 40	< 40	< 40

*Refer to Appendix B for the report of analysis.

2.4 Stability

Results of stability studies conducted on cranberry extract powder are provided in Table 2.4-1 and Figure 2.4-1. The results demonstrate that cranberry extract powder is stable for several years when kept under dry conditions at room temperature and below.

Table 2.4-1 Stability Data on Cranberry Extract Powder

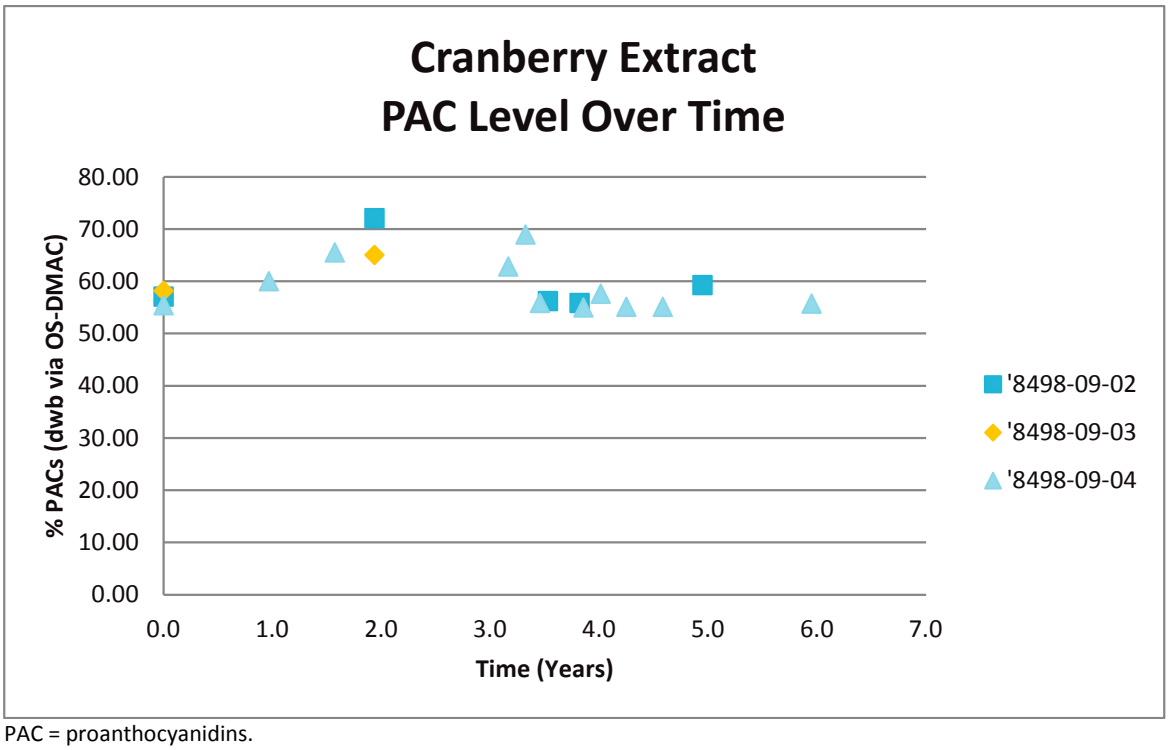
Lot Number	Age (Years)	PAC %dwb
8498-09-02	0.0	57.10
	1.9	72.09
	3.5	56.24
	3.9	55.86
	4.9	59.30
8498-09-03	0.0	58.16
	1.9	65.01
8498-09-04	0.0	55.45
	1.0	60.02
	1.6	65.53
	3.2	62.84
	3.3	68.96
	3.5	55.02
	3.9	55.82
	4.0	57.63

Table 2.4-1 Stability Data on Cranberry Extract Powder

	4.2	55.13
	4.6	55.13
	5.9	55.70
8498-12-01 E01:04	62.27	62.27
	57.35	57.35
	61.90	61.90
	61.16	61.16
	58.66	58.66
	56.81	56.81
	57.80	57.80
8498-14-02	0.0	50.80
	0.0	53.30
	0.7	54.78
	0.8	60.38

dwb = dry weight basis; PAC = proanthocyanidin.

Figure 2.4-1 Stability of Cranberry Extract Powder



PAC = proanthocyanidins.

Data to support the stability of cranberry extract powder following incorporation into beverages are provided in Table 2.4-2. The results indicate that cranberry extract powder is stable within diet- and water-type beverages for a period of up to 22 weeks from the time of production based on the level of proanthocyanidin and total phenolic content measured.

Table 2.4-2 Stability Data for Cranberry Extract Powder Following Incorporation into Beverages

Beverage Description	Time from Production (weeks)	PACs (mg/8 oz)[a]	Total Phenolics[b] (ppm)
Test beverage 40 mg (diet)	t=0	45.1	170
Test beverage 40 mg (water)	t=0	49	160
Test beverage 40 mg (diet)	t=2	48.7	160
Test beverage 40 mg (water)	t=2	49	110
Test beverage 40 mg (diet)	t=6	41.7	180
Test beverage 40 mg (water)	t=6	43.7	150
Test beverage 40 mg (diet)	t=10	42.7	130
Test beverage 40 mg (water)	t=10	48.7	110
Test beverage 40 mg (diet)	t=14	41.7	130
Test beverage 40 mg (water)	t=14	45.2	120
Test beverage 40 mg (diet)	t=18	39.2	140
Test beverage 40 mg (water)	t=18	41.9	100
Test beverage 40 mg (diet)	t=22	42	150
Test beverage 40 mg (water)	t=22	43	130

PACs = proanthocyanidins.

[a] The test method used was DMAC.

[b] The test method used was Folin Ciocalteau.

Part 3. §170.235 Dietary Exposure

3.1 Background Exposure

3.1.1 Background Exposure to Cranberries and Cranberry Juice

Cranberries are fruit native to North America and they have been consumed for hundreds of years, with the first commercial production reported to have begun in the 1840s (Kiple and Ornelas, 2000; Blumenthal, 2003). Existing cranberry juice beverages available in the market contain proanthocyanidins (*i.e.*, 576 mg per serving), phenolics (*i.e.,* 736 mg per serving), and anthocyanins (*i.e.*, 53 mg per serving) at levels much greater than the levels proposed for beverage use from cranberry extract powder (see Table 1.3-1). In the U.S., the estimated mean per-user daily intake of cranberries by male and female adults is 23.6 and 42.1 g, respectively, which is equivalent to 368 and 481 mg/kg body weight/day, respectively. The estimated 90th percentile per user daily intake of cranberries by male and female adults is 69.3 and 103.9 g/day, respectively (equivalent to 925 and 1,441 mg/kg body weight/day, respectively) (CDC, 2006, 2009; USDA, 2009). These intake results were based on food consumption data included in the National Center for Health Statistics (NCHS)'s National Health and Nutrition Examination Surveys (NHANES) (survey data from years 2003–2004, 2005–2006) (CDC, 2009; USDA, 2008, 2009).

Similarly, cranberry juice products have a long history of safe use. According to the NHANES of 2005–2008 (CDC, 2009; USDA, 2009, 2010), the mean per-user daily intake consumption of cranberry juice (100% cranberry juice, cranberry juice cocktail, low-calorie cranberry juice cocktail and blended cranberry drinks) was 221 mL. Duffey and Sutherland (2013) considered that the amount of cranberry juice in products to be around 27% due to the astringency of cranberry juice. Ocean Spray considers 'cranberry juice' consumption as recorded by NHANES represents beverages with about 27% cranberry juice and not the pure juice.

Target consumers' (*i.e.*, adults) consumption of cranberry beverage in the U.S. NHANES (2013–2014) (CDC, 2015, 2016; USDA, 2016), presented in Table 3.1.1-1 and 3.1.1-2 (per consumer only) and (per kg body weight, consumer only).

Table 3.1.1-1 Summary of the Estimated Daily Intake of Cranberry Beverage in the U.S. by Population Group (2013–2014 NHANES Data) (CDC, 2015, 2016; USDA, 2016)

Population Group	Age Group (Years)	Consumer-Only Intake (mL/day)					
		%	n	Mean	90th Percentile	95th Percentile	97.5th Percentile
Female Teenagers	12 to 19	1.5	11	227	394	445	471
Male Teenagers	12 to 19	1.7	4	247	228	316	441
Female Adults	20 and up	2.0	60	203	310	1,201	1,201
Male Adults	20 and up	1.5	30	166	314	379	521
Total Population	All ages	1.8	145	177	310	496	806

n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

Table 3.1.1-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Cranberry Beverage in the U.S. by Population Group (2013–2014 NHANES Data) (CDC, 2015, 2016; USDA, 2016)

Population Group	Age Group (Years)	Consumer-Only Intake (mL/kg bw/day)					
		%	n	Mean	90th Percentile	95th Percentile	97.5th Percentile
Female Teenagers	12 to 19	1.6	11	3.0	4.8	4.9	5.0
Male Teenagers	12 to 19	1.7	4	2.9	2.4	4.6	7.8
Female Adults	20 and up	2.1	60	2.2	5.0	7.4	7.4
Male Adults	20 and up	1.5	30	1.9	2.8	6.4	7.2
Total Population	All ages	1.8	145	2.9	7.2	7.7	9.3

bw = body weight; n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

3.1.2 Background Intake of Polyphenols

Since many foods are naturally rich in many of the polyphenols, their long history of safe consumption in food is well established (Erdman *et al.*, 2007). An accurate quantification of the dietary intake of polyphenols is greatly hindered by the lack of comprehensive food composition databases, despite recent development of polyphenol profiles of certain foods (Erdman *et al.*, 2007). Consequently, a wide range of dietary intake estimates for flavonoids were reported in peer-reviewed literature (Beecher, 2003, 2004; Prior and Gu, 2005; Erdman *et al.*, 2007). Beecher (2003) estimated the total flavonoids (flavones, flavonols, isoflavones) intake is in the range of 20 to 34 mg/day. Manach *et al.* (2005) cited the daily intake of flavonols alone as 20 to 35 mg/day. A review by Erdman *et al.* (2007) suggests that average dietary flavonoid intake in Western populations appears to be in the range of 65 to 250 mg/day, and in the American diet it is about 120 mg/day, with the majority coming from flavan-3-ols. In a recent publication, the average consumption of flavonoids from the diet of male and female U.S. adults was reported to be 207 mg/day (Chun *et al.*, 2010). Bentivegna and Whitney (2002) reported an average consumption of flavonoids from combined natural food sources such as fruits, vegetables, chocolate, and tea, in the range of 460 to 1,000 mg/day which would be equivalent to between 7.7 and 16.7 mg/kg body weight/day for a 60-kg individual. Using the United States Department of Agriculture (USDA) Database for Proanthocyanidin Content of Selected Foods (USDA PA Database) along with food consumption data from NHANES 1999–2002 CDC (2007), Wang *et al*. (2011) estimated the dietary intake of proanthocyanidins (PACs) and identified major sources of these compounds in the US diet. Total PAC intake was estimated to be 95 mg/day. Of this, 30% was in the form of polymers, 22% was in the form of monomers, 16% as dimers, 15% as 4 to 6 mers, 11% as 7 to 10 mers, and 5% as trimers. Three major food sources (*i.e.*, tea, legumes, and wines), contributed 45 mg (48%) of total daily PAC intake. Recently, Ziauddeen *et al*. (2018) estimate polyphenol intake in the United Kingdom (UK) population ranged from 266.6 ± 166.1 mg/day among children aged 1.5 to 3 years to 1,035.1 ± 544.3 mg/day in adults aged 65 years and over. Flavan-3-ols and hydroxycinnamic acids were the most commonly consumed (poly)phenols across all age groups, and non-alcoholic beverages and fruit served as the main food sources of polyphenols in the diet.

Anthocyanins are present in large amounts in some diets and the potential dietary intake of anthocyanins is among the greatest in the various classes of flavonoids (Wu *et al.*, 2006). The smallest intake estimate of anthocyanins, 1.3 mg/day for the U.S. population, was reported in a review paper by Erdman *et al.* (2007). Wu *et al.* (2006) estimated the daily intake of anthocyanins as 12.5 mg/day based on food consumption data from the NHANES 2001–2002 and levels of anthocyanins present in 24 fruits, vegetables, and nuts foods (CDC, 2007). Servings of 200 g of aubergine or black grapes can provide up to 1,500 mg anthocyanins, and servings of 100 g of berries up to 500 mg. Therefore, an intake of several hundred milligrams would not be considered exceptional (Manach *et al.,* 2005). In an earlier study, the average daily intake of total anthocyanins was estimated to be 215 mg/day during the summer and 180 mg/day during the winter (Kühnau, 1976). Since the levels of anthocyanins are consistently similar between various published studies, Wu *et al.* (2006) attributed the differences between their low estimates as compared to earlier higher estimates to different food intake data that were relied upon to estimate intake of anthocyanins. The mean dietary intake in Finland has been estimated to be 82 mg/day, with the main sources being berries, red wine, juices, and the coloring agent E163 (M. Heinonen, personal communication, 2001, as cited in Manach *et al.,* 2005). The daily intake of anthocyanins in Germany was estimated to be varied between 0 and 76 mg/day (Watzl *et al.,* 2002). Based on the high levels of anthocyanins present in fruits and vegetables, intakes of >100 mg/day could be achieved with regular consumption of select fruits or berries, such as blackberries, raspberries, blueberries, or Concord grapes (Wu *et al.*, 2006).

3.2 Estimated Intake of Cranberry Extract Powder

3.2.1 Methods

An assessment of the anticipated intake of cranberry extract powder as an ingredient under the intended conditions of use (see Table 1.3-1) was conducted using data available in the 2013–2014 cycle of the U.S. NCHS's NHANES (CDC, 2015, 2016; USDA, 2016). A detailed description of the survey and methodology employed in the intake assessment of cranberry extract powder is provided in Appendix C while an abbreviated summary along with the pertinent results is presented herein.

The NHANES data are collected and released in 2-year cycles with the most recent cycle containing data collected in 2013–2014. Information on food consumption was collected from individuals *via* 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2). Sample weights were incorporated with NHANES data to compensate for the potential under-representation of intakes from specific populations and allow the data to be considered nationally representative (CDC, 2015, 2016; USDA, 2016). The NHANES data were employed to assess the mean and 90th percentile intake of cranberry extract powder for each of the following population groups:

- Infants and young children, up to and including 2 years;
- Children, ages 3 to 11;
- Female teenagers, ages 12 to 19;
- Male teenagers, ages 12 to 19;
- Female adults, ages 20 and up;
- Male adults, ages 20 and up; and
- Total population (all age and gender groups combined).

Consumption data from individual dietary records, detailing food items ingested by each survey participant, were collated by computer and used to generate estimates for the intake of cranberry extract powder by the U.S. population . Estimates for the daily intake of cranberry extract powder represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES 2013–2014 (CDC, 2015, 2016; USDA, 2016); these average amounts comprised the distribution from which mean and percentile intake estimates were determined. Mean and percentile estimates were generated incorporating survey weights in order to provide representative intakes for the entire U.S. population. "*Per capita*" intake refers to the estimated intake of cranberry extract powder averaged over all individuals surveyed, regardless of whether they consumed food products in which cranberry extract powder is proposed for use, and therefore includes individuals with "zero" intakes (*i.e.*, those who reported no intake of food products containing cranberry extract powder during the 2 survey days). "Consumer-only" intake refers to the estimated intake of cranberry extract powder by those individuals who reported consuming food products in which the use of cranberry extract powder is currently under consideration. Individuals were considered "consumers" if they reported consumption of 1 or more food products in which cranberry extract powder is proposed for use on either Day 1 or Day 2 of the survey.

The estimates for the intake of cranberry extract powder was generated using the maximum use-level indicated for each intended food-use, as presented in Table 1.3-1, together with food consumption data available from the 2013–2014 NHANES datasets (CDC, 2015, 2016; USDA, 2016). The results for these assessments are presented in Section 3.2.

3.2.2 Intake Estimates for Cranberry Extract Powder

A summary of the estimated daily intake of cranberry extract powder from proposed food-uses is provided in Table 3.2.2-1 on an absolute basis (mg/person/day), and in Table 3.2.2-2 on a body weight basis (mg/kg body weight/day).

The percentage of consumers was low among all age groups evaluated in the current intake assessment; ranging from 22.6% to 55.6% of the population groups consisted of users of those beverage products in which cranberry extract powder is currently proposed for use (Table 3.2.2-1). Children had the greatest proportion of consumers at 55.6%. The consumer-only estimates are more relevant to risk assessments as they represent exposures in the target population. Consequently, only the consumer-only intake results are discussed in detail.

Among the total population (all ages), the mean and 90th percentile consumer-only intakes of cranberry extract powder were determined to be 332 and 639 mg/person/day, respectively. Of the individual population groups, male teenagers were determined to have the greatest mean at 449 mg/person/day, while male adults were determined to have the greatest 90th percentile consumer-only intakes of cranberry extract powder on an absolute basis at 856 mg/person/day, respectively. Infants and young children were identified to have the lowest mean and 90th percentile consumer-only intakes of 242 and 486 mg/person/day, respectively (Table 3.2.2-1).

Table 3.2.2-1 Summary of the Estimated Daily Intake of Cranberry Extract Powder from Proposed Food-Uses in the U.S. by Population Group (2013–2014 NHANES Data) (CDC, 2015, 2016; USDA, 2016)

Population Group	Age Group (Years)	*Per Capita* Intake (mg/day)		Consumer-Only Intake (mg/day)			
		Mean	90th Percentile	%	N	Mean	90th Percentile
Infants and Young Children	0 to 2	55	194	22.6	142	242	486
Children	3 to 11	147	407	55.6	721	263	518
Female Teenagers	12 to 19	148	388	46.4	281	319	590
Male Teenagers	12 to 19	211	504	46.9	274	449	808
Female Adults	20 and up	100	317	31.5	719	316	634
Male Adults	20 and up	113	350	30.1	673	375	856
Total Population	All ages	116	354	35.1	2,810	332	639

n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

On a body weight basis, the total population (all ages) mean and 90th percentile consumer-only intakes of cranberry extract powder were determined to be 5.8 and 12.9 mg/kg body weight/day, respectively. Among the individual population groups, infants and young children were identified as having the highest mean and 90th percentile consumer-only intakes of any population group, of 20.0 and 35.2 mg/kg body weight/day, respectively. Female adults had the lowest mean and 90th percentile consumer-only intakes of 4.2 and 8.8 mg/kg body weight/day, respectively (Table 3.2.2-2). However, the target population is the adult general population; the cranberry extract powder is not intended to be marketed to infants, toddlers and children of below 19 years of age.

Table 3.2.2-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Cranberry Extract Powder from Proposed Food-Uses in the U.S. by Population Group (2013–2014 NHANES Data) (CDC, 2015, 2016; USDA, 2016)

Population Group	Age Group (Years)	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants and Young Children	0 to 2	4.5	15.6	22.7	142	20.0	35.2
Children	3 to 11	5.3	15.1	55.5	716	9.5	18.1
Female Teenagers	12 to 19	2.4	6.6	46.3	277	5.1	10.6
Male Teenagers	12 to 19	3.0	7.3	47.2	273	6.4	13.1
Female Adults	20 and up	1.3	4.3	31.6	716	4.2	8.8
Male Adults	20 and up	1.3	4.2	30.2	671	4.3	9.1
Total Population	All ages	2.0	5.9	35.1	2,795	5.8	12.9

bw = body weight; n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

3.3 Results of Intake Estimates for Polyphenols

A summary of the estimated daily intake of polyphenols (*i.e.,* proanthocyanidins, anthocyanins, phenolics and organic acids) from cranberry extract powder from proposed food-uses is provided in Table 3.3-1 on an absolute basis (mg/person/day), and in Table 3.3-2 on a body weight basis (mg/kg body weight/day).

Among the total population (all ages), the mean and 90th percentile consumer-only intakes of proanthocyanidins from cranberry extract powder were determined to be 177 and 340 mg/person/day, respectively. Of the individual population groups, male teenagers were determined to have the greatest mean at 239 mg/person/day, while male adults were determined to have the greatest 90th percentile intakes of proanthocyanidins from cranberry extract powder on an absolute basis at 456 mg/person/day, respectively. Infants and young children had the lowest mean and 90th percentile consumer-only intakes of 129 and 259 mg/person/day, respectively (Table 3.3-1).

Among the total population (all ages), the mean and 90th percentile intakes of anthocyanins from cranberry extract powder were determined to be 27 and 51 mg/person/day, respectively. The equivalent intakes of phenolics were 135 and 260 mg/kg body weight/day, respectively; while organic acid intakes from this ingredient were 2.1 and 4.1 mg/kg body weight/day, respectively.

Of the individual population groups, male teenagers were determined to have the greatest consumer-only mean intakes for anthocyanins, phenolics, and organic acids on an absolute basis of 36, 182, and 2.9 mg/person/day, respectively. At the 90th percentile, male adults were determined to have the greatest 90th percentile consumer-only intakes of anthocyanins, phenolics, and organic acids from cranberry extract powder at 69, 348, and 5.5 mg/person/day, respectively.

Table 3.3-1 Summary of the Estimated Daily Intake of Polyphenols – Proanthocyanidins, Anthocyanins, Phenolics and Organic acids from Cranberry Extract Powder from Proposed Food-Uses by Consumers Only in the U.S. by Population Group (2013–2014 NHANES Data) (CDC, 2015, 2016; USDA, 2016)

Population Group	Age Group (Years)	%(n)	Proanthocyanidins (mg/day)		Anthocyanins (mg/day)		Phenolics (mg/day)		Organic acids (mg/day)	
			Mean	P90	Mean	P90	Mean	P90	Mean	P90
Infants and Young Children	0 to 2	22.6 (142)	129	259	19	39	98	197	1.6	3.1
Children	3 to 11	55.6 (721)	140	276	21	41	107	211	1.7	3.3
Female Teenagers	12 to 19	46.4 (281)	170	314	25	47	129	240	2.0	3.8
Male Teenagers	12 to 19	46.9 (274)	239	431	36	65	182	328	2.9	5.2
Female Adults	20 and up	31.5 (719)	168	338	25	51	128	258	2.0	4.1
Male Adults	20 and up	30.1 (673)	200	456	30	69	152	348	2.4	5.5
Total Population	All ages	35.1 (2,810)	177	340	27	51	135	260	2.1	4.1

n = sample size; NHANES = National Health and Nutrition Examination Survey; P90 = 90th percentile; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

On a body weight basis, the total population (all ages) mean and 90th percentile intakes of proanthocyanidins from cranberry extract powder were determined to be 3.1 and 6.9 mg/kg body weight/day, respectively (Table 3.3-2). Among the individual population groups, infants and young children were identified as having the highest mean and 90th percentile proanthocyanidin intakes of any population group, of 10.7 and 18.8 mg/kg body weight/day, respectively. Female adults had the lowest mean and 90th percentile consumer-only intakes of 2.2 and 4.7 mg/kg body weight/day, respectively.

The total population (all ages) mean and 90th percentile intakes of anthocyanins from cranberry extract powder were determined to be 0.5 and 1.0 mg/kg body weight/day, respectively. The equivalent intakes of phenolics were 2.4 and 5.3 mg/kg body weight/day, respectively; while organic acid intakes from this ingredient were 0.04 and 0.08 mg/kg body weight/day, respectively.

Among the individual population groups, infants and young children were identified as having the highest mean consumer-only intakes of anthocyanins, phenolics and organic acids at 1.6, 8.1, and 0.13 mg/kg body weight/day, respectively. This age group was also determined to have the highest 90th percentile intakes of anthocyanins, phenolics and organic acids at 2.8, 14.3, and 0.23 mg/kg body weight/day, respectively.

Table 3.3-2 Summary of the Estimated Daily Intake of Polyphenols – Proanthocyanidins, Anthocyanins, Phenolics and Organic acids from Cranberry Extract Powder from Proposed Food-Uses by Consumers Only in the U.S. by Population Group (2013–2014 NHANES Data) (CDC, 2015, 2016; USDA, 2016)

Population Group	Age Group (Years)	%(n)	Proanthocyanidins (mg/kg bw/day)		Anthocyanins (mg/kg bw/day)		Phenolics (mg/kg bw/day)		Organic acids (mg/kg bw/day)	
			Mean	P90	Mean	P90	Mean	P90	Mean	P90
Infants and Young Children	0 to 2	22.7 (142)	10.7	18.8	1.6	2.8	8.1	14.3	0.13	0.23
Children	3 to 11	55.5 (716)	5.1	9.7	0.8	1.5	3.9	7.4	0.06	0.12
Female Teenagers	12 to 19	46.3 (277)	2.7	5.6	0.4	0.8	2.1	4.3	0.03	0.07
Male Teenagers	12 to 19	47.2 (273)	3.4	7.0	0.5	1.0	2.6	5.3	0.04	0.08
Female Adults	20 and up	31.6 (716)	2.2	4.7	0.3	0.7	1.7	3.6	0.03	0.06
Male Adults	20 and up	30.2 (671)	2.3	4.9	0.3	0.7	1.7	3.7	0.03	0.06
Total Population	All ages	35.1 (2,795)	3.1	6.9	0.5	1.0	2.4	5.3	0.04	0.08

n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

Estimated PAC consumption by target consumers (*i.e.*, adults) from the proposed uses of cranberry extract powder are compared to the PAC intake resulting from consumption of 27% cranberry juice are shown in Table 3.3-3.

Table 3.3-3 Comparison of Proanthocyanidins Intake from Proposed Conditions of Use of Cranberry Extract Powder to Intake from 27% Cranberry Juice

Female Adults	168	338	132	202	2.2	4.7	1.43	3.25
Male Adults	200	456	108	204	2.3	4.9	1.24	1.82

P90 = 90th percentile.

3.4 Summary and Conclusions

Consumption data and information pertaining to the intended food-uses of cranberry extract powder were used to estimate the *per capita* and consumer-only intakes of this ingredient for specific demographic groups and for the total U.S. population. There were a number of assumptions included in the assessment which render exposure estimates suitably conservative. For example, it has been assumed in this exposure assessment that all food products within a food category contain cranberry extract powder at the maximum specified level of use. In reality, the levels added to specific foods will vary depending on the nature of the food product and it is unlikely that cranberry extract powder will have 100% market penetration in all identified food categories.

In summary, on a consumer-only basis, the resulting mean and 90th percentile intakes of cranberry extract powder by the total U.S. population from all proposed food-uses, were estimated to be 332 mg/person/day (5.8 mg/kg body weight/day) and 639 mg/person/day (12.9 mg/kg body weight/day), respectively. Among the individual population groups, male teenagers were determined to have the greatest mean at 449 mg/person/day (6.4 mg/kg body weight/day), while male adults were determined to have the greatest 90th percentile consumer-only intakes of cranberry extract powder on an absolute basis at 856 mg/person/day (9.1 mg/kg body weight/day), respectively. Infants and young children had the lowest mean and 90th percentile consumer-only intakes of 242 and 486 mg/person/day, respectively. When intakes were expressed on a body weight basis, infants and young children had the highest mean and 90th percentile consumer-only intake of 20.0 and 35.2 mg/kg body weight/day, respectively. Female adults had the lowest mean and 90th percentile consumer-only intakes of 4.2 and 8.8 mg/kg body weight/day, respectively.

In summary, on consumer-only basis, the resulting mean and 90th percentile intakes of proanthocyanidins from cranberry extract powder by the total U.S. population from all proposed food-uses, were estimated to be 177 mg/person/day (3.1 mg/kg body weight/day) and 340 mg/person/day (6.9 mg/kg body weight/day), respectively. Among the individual population groups, male teenagers were determined to have the greatest mean at 239 mg/person/day (3.4 mg/kg body weight/day), while male adults were determined to have the greatest 90th percentile consumer-only intakes of proanthocyanidins from cranberry extract powder on an absolute basis at 456 mg/person/day (4.9 mg/kg body weight/day), respectively. When intakes were expressed on a body weight basis, infants and young children had the highest mean and 90th percentile consumer-only intake of 10.7 and 18.8 mg/kg body weight/day, respectively. Female adults had the lowest mean and 90th percentile consumer-only intakes of 2.2 and 4.7 mg/kg body weight/day, respectively.

Among the total population (all ages), the mean and 90th percentile intakes of anthocyanins from cranberry extract powder were determined to be 27 mg/person/day (0.5 mg/kg body weight/day) and 51 mg/person/day (1.0 mg/kg body weight/day), respectively. The equivalent intakes of phenolics were 135 and 260 mg/person/day (2.4 and 5.3 mg/kg body weight/day), respectively; while organic acid intakes from this ingredient were 2.1 and 4.1 mg/person/day (0.04 and 0.08 mg/kg body weight/day), respectively.

Part 4. §170.240 Self-Limiting Levels of Use

The use cranberry extract powder would be self-limiting due to its organoleptic properties (*i.e.,* bitter and astringent taste).

Part 5. §170.245 Experience Based on Common Use in Food Before 1958

Not applicable.

Part 6. §170.250 Narrative and Safety Information

6.1 Introduction

To obtain the necessary information, comprehensive and detailed searches of the published scientific literature were conducted through January 2018. AdisInsight: Trials, AGRICOLA, AGRIS, Allied & Complementary Medicine™, BIOSIS® Toxicology, BIOSIS Previews®, CAB ABSTRACTS, Embase®, Foodline®: SCIENCE, FSTA®, MEDLINE®, NTIS: National Technical Information Service, and ToxFile® served as the primary sources of published literature pertinent to the safety of cranberry extract powder.

Although cranberry juices and extracts are widely consumed all over the world, few traditional assessments of safety have been evaluated. The absorption, distribution, metabolism, and excretion of cranberry constituents are summarized in Section 6.2. The safety of various cranberry extracts was evaluated at an average dose of up to 0.60 mg/kg body weight/day of phenolic compounds following dietary administration to 10-week-old male Wistar rats for a period of 14 weeks (Palikova *et al*., 2010). As cranberry preparations have been used as an adjunctive treatment for urinary tract infections, the effects of cranberry juice and extract consumption have been widely studied in human studies. Details are provided in Section 6.3.1. In addition, as discussed in Part 4, a comparison of these levels of intake resulting from the proposed uses of cranberry extract powder to those already in the diet demonstrate that these intakes are not expected to be a safety concern.

The safety of cranberry extract powder is also supported by the safety data available on its constituents (*e.g*., proanthocyanidins, anthocyanins, and other phenolics). Toxicity assessments of proanthocyanidin-rich grape seed extract (GSE) include an acute toxicity study and 3 sub-chronic toxicity studies (two 90-day and one 6-month study) in mice and rats, as well as genotoxicity and mutagenicity studies (an Ames bacterial mutagenicity assay, an *in vitro* chromosome aberration assay in Chinese hamster lung cells, and an *in vivo* mouse micronucleus study). Summaries of these studies are provided in Section 6.3.2.

6.2 Absorption, Distribution, Metabolism, and Excretion

There is considerable structural diversity among polyphenols that greatly influences their bioavailability. For some of the compounds, bioavailability can vary depending on dietary sources and on the forms they contain. Phenolic acids like caffeic acid are easily absorbed through the gut barrier, whereas large molecular weight polyphenols such as proanthocyanidins polymers are very poorly absorbed. Flavan-3-ols, flavonols, and anthocyanins are bioavailable to various degrees. Scalbert and Williamson (2000) reported that plasma concentration of any individual polyphenol rarely exceeds 1 µM after the consumption of 10 to 100 mg of a single compound. Manach *et al.* (2005) reviewed data from 97 studies that investigated the kinetics and extent of polyphenol absorption among adults, after ingestion of a single dose of polyphenol provided as pure compound, plant extract, or whole food/beverage. These authors concluded that gallic acid and isoflavones are the well-absorbed polyphenols, followed by catechins, flavanones, and quercetin glucosides, but with different kinetics. Proanthocyanidins, the galloylated tea catechins, and the anthocyanins are the least well absorbed polyphenols. The data below in Table 6.2-1 were converted to correspond to the same supply of polyphenols, a single 50 mg dose of aglycone equivalent.

Table 6.2-1 Summary of Pharmacokinetic Data from Bioavailability Studies

Compounds	Mean C_{max} (µmol/L)	Mean Urinary Excretion (% of intake)	Mean Half-Life (h)
Catechins	~1.6	10–20	2–3
Flavanones	0.50	9	2
Quercetin glucosides	1.5	2.5	18
Proanthocyanidins	0.02	ND	ND
Anthocyanins	0.03	0.4	ND

C_{max} = maximal concentration in plasma; h = hour(s); ND = not determined.

Once absorbed, polyphenols are conjugated to glucuronide, sulphate, and methyl groups in the gut mucosa and systemic tissues. Non-conjugated polyphenols are virtually absent in plasma. Such reactions facilitate their excretion and limit their potential toxicity. The polyphenols reaching the colon are extensively metabolized by the microflora into a wide array of low molecular weight phenolic acids. The biological properties of both conjugated derivatives and microbial metabolites have rarely been examined. Some health effects of polyphenols may not require their absorption through the gut barrier (Scalbert *et al.*, 2002).

Anthocyanin bioavailability appears to be very low. The literature includes numerous studies in which single doses of 150 mg to 2 g total anthocyanins were given to volunteers in the form of berries, berry extract, or concentrates. The measured plasma concentration after intake was very low (10 to 50 nmol/L). However, Manach *et al.* (2005) has noted that absorption could have been underestimated since important metabolites of anthocyanins may have been ignored and/or analytical methods may not be optimal.

Flavonols have been extensively studied because they are widely distributed in dietary plants. Available evidence has shown that bioavailability for quercetin differs among food sources, depending on the type of glycosides they contain. For example, onions, which contain glucosides are better sources of bioavailable quercetin than are apples and tea, which contain rutin and other glycosides (Manach *et al.,* 2005). Some phenolic and aromatic acids can also be produced from flavonols by the microflora. One characteristic feature of quercetin bioavailability is that the elimination of quercetin metabolites is quite low, with reported half-lives ranging from 11 to 28 hours. This could favor accumulation in plasma with repeated intakes. Volunteers in these studies consumed flavonol-containing foods such as onions, apples, buckwheat tea, or apple cider or pure flavonols such as quercetin, rutin, or quercetin glucosides. Doses ranged from 2 to 200 quercetin equivalents (Manach *et al.*, 2005).

Very little is known about the metabolic fate of proanthocyanidins (Prior and Gu, 2005; Williamson and Manach, 2005). Because some proanthocyanidins are very high in molecular weight, they are unlikely to be absorbed intact. Detection of proanthocyanidins dimers B1 and B2 in human plasma was reported in only 2 studies and absorption was minor. However, effects of proanthocyanidins may not require efficient absorption through the gut. These compounds may have a direct effect on the intestinal mucosa and protect it against oxidative stress or the actions of carcinogens. Thus, gut metabolism may have a major role in their physiological effect (Manach *et al.,* 2005; Prior and Gu, 2005).

Proanthocyanidins are present in 43 foods, including 23 kinds of fruits, 7 nuts, 8 cereals/beans, 2 beverages, 2 spices, and 1 vegetable (Prior and Gu, 2005). Structural differences of proanthocyanidins in various foods, such as differences in molecular size, interflavan linkages, and hydroxylation pattern on the constituent flavan-3-ols, has been shown to affect their metabolism and biological effects (*e.g.*, inhibition of uropathogenic P-fimbriated *Escherichia coli* adherence to cellular surfaces *in vitro*; transcription of interleukin-2 in peripheral blood mononuclear cells; antioxidant activity; anti-hypercholesterolemic effect *via* enhanced reverse cholesterol transport and reduction of intestinal cholesterol absorption and increased

bile acid excretion) (Prior and Gu, 2005; Neto, 2007). Typically, proanthocyanidin molecules contain 2 types of linkages between epicatechin units: (i) the B-type linkage commonly reported in food sources such as apples, grape seed, cocoa; and (ii) the less common A-type linkage, reported in cranberry, peanut, plum, avocado and curry (Prior and Gu, 2005; Neto, 2007). Cranberry proanthocyanidins are primarily dimers, trimers, and larger oligomers of epicatechin. Sprygin and Kushnerova (2004) showed that a comparison of the percentage composition of the main fractions of complexes of oligomeric proanthocyanidins (COPCs) extracted from cranberry oilcake and grape seeds are highly similar. Neto (2007) suggests that it is reasonable to expect that the biological properties of cranberry proanthocyanidins will resemble those of other condensed tannins in some ways but differ in others, depending on structural similarity and also on how these compounds are metabolized *in vivo* (Neto, 2007). Given the general lack of toxicity data for flavonoids, a prudent approach to evaluating the safety of proanthocyanidins from cranberry would be to utilize the available toxicity data for proanthocyanidins from GSE (GSE proanthocyanidins content, 74 to 81%; cranberry extract powder proanthocyanidins content is 55%).

Absorption and excretion of twenty cranberry-derived phenolics were studied following the consumption of cranberry juice, sauces, and dried fruits by healthy human volunteers (6 females and 5 males, age 21 to 33 years). Plasma and urine samples were collected and analyzed by gas chromatography–mass spectrometry (GC–MS). Following consumption of the cranberry products, significant increases in the sum of plasma phenolics were observed with different concentration peaks (between 0.5 and 2 hours) for individual subjects.

Plasma samples, without and with hydrolysis was analyzed to compare free and bound compounds in biological fluids. Only 4 benzoic and phenolic acids (*e.g.*, benzoic, p-hydroxybenzoic, vanillic, and p-coumaric acids) were identified in plasma samples without hydrolysis. In addition to these acids, 6 additional compounds including o-hydroxybenzoic, trans-cinnamic, m-hydroxybenzoic, p-hydroxyphenylacetic, and o-phthalic acids and catechin were identified in the same samples after hydrolysis. The concentrations of 4 identified phenolic and benzoic acids in plasma samples increased after hydrolysis, indicating the contents of bound phenolic compounds were dominant in human plasma. Orally administered phenolics are absorbed from the stomach and these compounds reached maximum concentrations within an hour of ingestion. However, some of the phenolics, such as trans-cinnamic, vanillic, p-coumaric acids, and catechin showed second plasma concentration peaks, potentially due to the reabsorption of biliary excreted phenolics from the jejunum. All of cranberry-derived phenolics increased significantly in urine samples after the intake of each cranberry product. Furthermore, urinary levels were higher than those in plasma, suggesting a lack of accumulation in organs or tissues during their absorption and metabolism. However, the high molecular weight quercetin and myricetin, found in cranberry foodstuffs, were not found in either plasma or urine samples. Myricetin, which were abundant in cranberry foodstuffs, were not found in plasma, indicating a lower bioavailability of these flavonoids compared to simple phenolics (Wang *et al*., 2012a). In contrast, Iswaldi *et al*. (2013) were able to detect 3 flavonols, namely methoxyquercetin 3-O-galactoside, myricetin, and quercetin, in the urine of humans after a single dose of cranberry syrup.

Wang *et al*. (2016) measured the urinary excretion of cranberry flavonoids by 10 healthy female adults at 90, 225, 360 minutes following ingestion of 240 mL of cranberry juice. While no flavan-3-ol monomers or dimers were detected, the parent flavonol glycosides (*i.e*., myricetin-3-arabinoside, myricetin-3-galactoside, quercetin-3-galactoside, quercetin-3-arabinoside and quercetin-3-rhamnoside were detected in urine. Peak excretion levels for most glycosides was seen 1.5 to 2.5 hours after ingression; quercetin-3-arabinoside was an exception, with the highest excretion observed after 4.0 hours. The authors speculated that the high inter-individual variability in excretion levels was due to gastric-intestinal variability for intestinal transporter, enzyme or microbiota activity. Quercetin-3-galactoside was the most abundant cranberry

flavonol, and exhibited the highest peak excretion levels. The sugar moiety also affected the efficiency of flavonol absorbance efficiency. Despite being present at lower concentrations in the cranberry juice, myricetin-3-arabinoside and quercetin-3-rhamnoside were detected at higher levels in the urine than myricetin-3-galactoside and quercetin-3-arabinoside. These findings indicate that the major cranberry flavonol glycosides can be absorbed into the human circulatory system and excreted through urine in intact forms (Wang *et al*., 2016).

McKay *et al.* (2015) demonstrated that phenolic compounds in cranberry juice are bioavailable and exert antioxidant actions in healthy older adults. Ten healthy, nonsmoking men and postmenopausal women (age 50 to 70 years) consumed a low-calorie cranberry juice cocktail (54% juice, containing 188.5 mg phenolics). Blood samples were collected *via* an indwelling catheter at 0.25, 0.5, 1 to 6, and 10 hours following consumption of the juice and 24-hour urine samples were assessed for phenolic acids, flavonoids (including flavonols, flavanols, anthocyanins, and proanthocyanidins). Plasma total antioxidant capacity was evaluated. Authors analyzed the pharmacokinetic profile of 13 phenolic acids, 2 flavanols, 4 flavonols, 6 anthocyanins, 4 anthocyanin glucuronides, and 1 PAC. The maximum concentration of cranberry juice cocktail anthocyanins were typically reached in plasma within 3 hours and in urine within 4 hours of consumption. A bimodal distribution of peak plasma concentrations was observed for all of the flavanols and 7 phenolic acids. Caffeic acid exhibited a trimodal distribution. PAC-A2 was detectable in plasma and quantifiable in urine. Increase antioxidant capacity was also observed. The maximal concentration in plasma (C_{max}) and time to maximal concentration (T_{max}) exhibited inter-individual variability in both plasma and urine. The authors attributed this to individual differences in phase II enzyme polymorphisms, as well as composition of gastrointestinal (GI) microbiota.

Nelson *et al*. (2016) investigated the bioavailability of cranberry flavonol glycosides. Healthy women (n=10) ingested low-acidity 27% v/v cranberry juice or isocaloric control. Urine was collected at baseline (0), then 90, 225, and 360 minutes postprandially. Although not detected at baseline, several glycosides (*i.e.*, quercetin-3-galactoside, quercetin-3-arabinoside, myricetin-3-galactoside, quercetin-3-rhamnoside, and myricetin-3-arabinoside) were found in urine postprandially. Sugar conjugate specificity at the 3-linkage appears to affect flavonol glycoside bioavailability, with higher levels of galactosides and rhamnosides detected compared to those found for arabinosides.

The absorption, metabolism, and excretion of cranberry (poly)phenols was analyzed in healthy young men (n=10) following consumption of a cranberry juice containing 787 mg polyphenols. A total of 60 metabolites were identified, including cinnamic acids, dihydrocinnamic, benzoic acids, phenylacetic acids, benzaldehydes, valerolactones, hippuric acids, catechols, and pyrogallols. Only 3 metabolites were flavonol derivatives (*i.e.*, kaempferol, kaempferol-3-O-ß-D-glucuronide, and quercetin-3-O-ß-D-glucuronid), while the remainder consisted of conjugated and non-conjugated derivatives of phenolic acids. The C_{max} for each of the metabolites were low, in the nanomolar and low micromolar range, and the authors speculated that many of these metabolites were derived from interaction with gut microbiota. After 24 hours, 6.2% of the consumed polyphenols had been collected in the urine. The metabolic profile of the urine was generally similar to that of plasma (Feliciano *et al*., 2016).

In a follow up study, Feliciano *et al.* (2017) evaluated the effect of dose on absorption, metabolism, and excretion. Ten healthy men consumed cranberry juices containing 409, 787, 1,238, 1,534 and 1,910 mg total (poly)phenols. The plasma levels of the same 60 metabolites were analyzed. Total plasma polyphenols exhibited a linear dose response, although the same linear dose-dependence was only seen for 14 individual metabolites. Likewise, excretion of 12 of the 60 metabolites exhibited a linear dose response. However, total excretion was non-linear, plateauing at intakes above 787 mg. The authors also reported inter-individual variability in the plasma metabolite concentration, which was dependent on the individual

metabolite. The authors speculated that this variation was due to differences in the gut microbiome involved in the metabolism of the phenolic components of the cranberry juice.

Kimble *et al*. (2018) evaluated the bioavailability of phenolic acids and flavonoids following acute or repeated consumption (once a day for 8 weeks) of a high polyphenol cranberry extract beverage (CEB) in healthy overweight individuals (30 to 70 years; n=78) with abdominal adiposity. Urinary levels of epicatechin, caffeic, p-coumaric, ferulic, sinapic and 4-OH-3-MeOH-phenylacetic acids were significantly increased following a single dose and after 8 week intervention. Quercetin and 3,4-OH-phenylacetic acid were significantly elevated after 8 week intervention. Similarly, concentrations of 6 anthocyanins (*i.e.*, galactosides, glucosides, arabinosides of cyanidin and peonidin) were significantly elevated after both single and repeated consumption compared to baseline levels. Proanthocyanidin A2 also increased after repeated ingestion ($P<0.05$) in 24-hour samples compared to placebo; epicatechin, gentisic, caffeic, p-coumaric, ferulic, sinapic, 4-OH-3-MeOH-phenylacetic and 3,4-OH-phenylacetic acids were also elevated ($P<0.05$) in 24-hour samples compared to baseline. These data demonstrate that cranberry phenolic acids and flavonoids are bioavailable in healthy overweight humans.

6.2.1.1 Summary

Proanthocyanidins and the anthocyanins, the primary polyphenols present in cranberry extract powder, are less bioavailable than other dietary polyphenols (*e.g*., isoflavones, catechins, flavanones). Differences in molecular size, interflavan linkages, and hydroxylation pattern on the constituent flavan-3-ols, influence the metabolism and biological effects of proanthocyanidins. As reported in the scientific literature, cranberry flavonol glycosides are absorbed into the human circulatory system. Once absorbed, polyphenols are conjugated to facilitate excretion. Urinary levels were higher than those in plasma, suggesting a lack of accumulation in organs or tissues during their absorption and metabolism. The polyphenols reaching the colon are extensively metabolized by the microflora into a wide array of low molecular weight phenolic acids. The extent and rate of absorption varies among individuals, likely due to individual differences in phase II enzyme polymorphisms as well as composition of GI microbiota.

6.3 Toxicological Studies

6.3.1 Cranberry Juice and Extract Powder

Cranberries are a fruit native to North America that has been consumed for hundreds of years with the first commercial production reported to have begun in the 1840s (Kiple and Ornelas, 2000; Blumenthal, 2003). In the U.S., the estimated mean per-user daily intake of cranberries by male and female adults is 23.6 and 42.1 g, respectively, which is equivalent to 368 and 481 mg/kg body weight/day, respectively. Heavy male and female adult consumers (90^{th} percentile) consume 69.3 and 103.9 g/day, respectively, which is equivalent to 925 and 1,441 mg/kg body weight/day, respectively (CDC, 2006, 2009; USDA, 2009). These intake estimates were based on food consumption data included in the NCHS NHANES (survey data from years 2003–2004, 2005–2006) (USDA, 2008, 2009; CDC, 2009). Cranberry juice has conditionally been recommended for individuals with urinary tract infections at consumption levels up to 500 mL/day or an equivalent dose of 600 to 1,200 mg/day of a concentrated juice extract (Blumenthal, 2003).

6.3.1.1 Preclinical Studies

Although cranberry juices and extracts are widely consumed, few traditional assessments of safety have been conducted. Parameters relating to the safety of various cranberry extracts were examined by Palikova *et al.* (2010) following dietary administration to 10-week-old male Wistar rats for a period of 14 weeks. The cranberry extracts included NUTRICRAN90S, a water-soluble spray-dried cranberry concentrate fruit juice, HI-PAC 4.0, a spray-dried cranberry fruit juice, and PACRAN, a cranberry powder consisting of 100% cranberry solids. A comparison of the phenolic composition of these extracts with that of the cranberry extract powder is presented in Table 6.3.1.1-1.

Table 6.3.1.1-1 Composition of Cranberry Extracts Examined by Palikova *et al.* (2010)

Compound	NutriCRAN90S (Group 2)	HI-PAC 4.0 (Group 3)	PACRAN (Group 4)	Cranberry Extract Powder
Proanthocyanidins	1.4	4.75	1.5	55 to 60
Organic Acids (%)	7.342	6.586	0.598	<1
Malonic acid	0.177	0.2	ND	-
Citric acid	1.98	1.954	ND	-
Malic acid	1.892	1.6	0.263	-
Quinic acid	2.57	2.085	ND	-
Phenolic Acids (%)				
Protocatechuic acid	0.065	0.077	0.052	-
Gentisic acid	0.008	0.023	ND	-
Caffeic acid	0.01	0.014	0.006	-
Dihydrocaffeic acid	0.121	0.129	0.053	-
Chlorogenic acid	0.025	0.035	ND	-
p-Coumaric acid	0.079	0.075	0.047	-
Vanillic acid	0.021	0.052	ND	-
Benzoic acid	0.372	0.311	0.16	-
Flavonoids (%)	1.352	2.179	0.68	4.0 to 10
Quercetin	1.352	2.179	0.68	0 to 2.0
Anthocyanins/anthocyanidins	0.44	0.61	0.105	2.0 to 10

ND = not determined.

The rats were randomized to 4 groups (n=6 per group) including the control groups and 1 group per cranberry extract diet (Palikova *et al.*, 2010). The diets were prepared containing 1,500 ppm of the cranberry extracts and were consumed *ad libitum* by the animals. All animals had free access to their respective diets and water. The feed consumption was checked twice a week. The health of the animals was checked daily and the body weight was monitored twice a week and prior to sacrifice. All animals were sacrificed at the end of the experimental period. On Day 100 following sacrifice, clinical chemistry [sodium, potassium, chlorides, bilirubin, cholesterol, urea, creatinine, and activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), cholinesterase (CHE), and total protein], and hematology parameters (hemoglobin, hematocrit, erythrocytes, mean volume of erythrocytes, thrombocytes, leukocytes, and differential leukocyte count) were examined. Histopathological examinations of the heart, liver, kidney, and ileum were conducted.

No significant differences in weight gain or organ weights were observed between the control and treatment groups. No clinical signs of toxicity were observed and the food consumption was not significantly different between animals consuming the control and cranberry extract-containing diets. The average doses of phenolic compounds were calculated to be 0.03 (control), 0.47 (Group 2), 0.72 (Group 3), and 0.60 mg/kg body weight/day (Group 4), respectively. The experimental diets did not significantly affect the monitored hematological, clinical chemistry parameters, or histopathological examinations. The components and metabolites of cranberry did not accumulate in the tissues and were excreted through the urine mainly as hippuric acid. In addition, to examine the genotoxicity of cranberry powders, formation of single-stranded DNA breaks in peripheral lymphocytes was analyzed by the Comet assay. Peripheral lymphocytes were isolated and DNA damage was analyzed using a fluorescence microscope, after staining with ethidium bromide. One hundred cells per slide were analyzed and divided into 4 classes of DNA damage standard scale (maximal damage set at a value of 400). The values obtained for Groups 1 to 4 were 3.8, 4.4, 2.3, and 9.7, respectively. There were no significant differences between groups. The authors reported that the tested commercial cranberry products (dry powdered juices and fruits) were well-tolerated by the animals.

Although this study was not conducted with Ocean Spray's material *per se*, the test materials would have contained very similar types, at least qualitatively, of polyphenolics relative to Ocean Spray's cranberry extract powder. Although the levels of proanthocyanins in these extracts listed in Table 6.3.1.1.-1 are shown to be much lower than those in Ocean's Spray's product, differences in the analytical data precluded a direct comparison with the cranberry extract powder. A number of analytical methodologies have been used in the past for quantifying the amount of PACs in a sample (*e.g.*, colorimetric, gravimetric, chromatographic and mass spectrometric techniques) (Prior *et al.,* 2010). However, these different methodologies produce highly variable results due to the complexity of the PAC structures and the A-type linkages present (Prior *et al.,* 2010). As such, comparisons of PAC content across products can be drawn only if they are quantified using the same methods and using the same standards. Since different methodologies of analysis and quantification were used in the Palikova publication (high performance liquid chromatography-mass spectrometry to that used by Ocean Spray (OSC-DMAC), it is therefore not possible to draw a direct comparison on the levels of PACs in Ocean Spray's product to that which was evaluated in the 3 commercially available products used within the Palikova *et al*. (2010) study.

In order to provide a more direct comparison, Ocean Spray re-analyzed their cranberry extract using the BL-DMAC method. In addition, Ocean Spray analyzed the PAC content of a variety of commercially available cranberry extracts currently marketed in France and throughout Europe. Results are presented in Table 6.3.1.1-2. The PAC content determined using the OSC-DMAC method typically used by Ocean Spray is also included for comparison purposes.

Table 6.3.1.1-2 Analysis of Polyphenol Content in Ocean Spray's Cranberry Extract and Other Currently Marketed Cranberry Extracts

	Total Anthocyanins (%)	PAC Content		Phenolics	ORAC (hydroFL)	Total Sugars (%)	Total Organic Acids (%)
		OSC-DMAC[a]	BL-DMAC[b]				
Ocean Spray's Cranberry Extract Powder	3.180	53.65	17.07	43.71	6,241	4.82	5.98
Commercially available cranberry extracts							
FutureCeuticals VitaCran	1.343	34.39	13.71	41.52	7,533	0.00	4.21
Pharmatoka Urell Capsules	1.357	32.59	13.26	26.70	3,892	0.00	2.29
FutureCeuticals CranForte	0.426	18.04	11.11	37.10	1,676	44.80	31.00
MB North America CranEx	0.535	24.04	9.89	35.84	3,561	0.00	1.18

Table 6.3.1.1-2 Analysis of Polyphenol Content in Ocean Spray's Cranberry Extract and Other Currently Marketed Cranberry Extracts

	Total Anthocyanins (%)	PAC Content		Phenolics	ORAC (hydroFL)	Total Sugars (%)	Total Organic Acids (%)
		OSC-DMAC[a]	BL-DMAC[b]				
Decas Cysticran	0.467	5.87	9.80	23.85	3,025	12.48	8.79
Cranberry Juice							
Cranberry juice cocktail	0.35	3.4	1.2	2.91	403	50.51	39.72

[a] Expressed as mg of cranberry specific PACs equivalents per 100 mg of powder.
[b] Expressed as mg of procyanidin A-2 or procyanidin B-2 dimer equivalents per 100 mg of powder.
All (%) values reported on dry weight basis (dwb).

The fact that all of these different cranberry extract products contain similar levels of PAC provides clarification that the 3 commercially available extracts used in the Palikova study likely also had a similar PAC level and that the differences were due solely to the different methodology and standards used. The similarity in the levels of PACs therefore enables the Ocean Spray's cranberry extract powder to be considered substantially equivalent to those other cranberry extract products currently on the market. The high level of PACs present within these different commercially available cranberry extract products can therefore be considered to be of no toxicological concern. As such the results of Palikova *et al*. (2010) corroborate the safety of Ocean Spray's product.

6.3.1.2 Human Studies

6.3.1.2.1 Studies in Adults

A randomized, double-blinded, placebo-controlled 10-week study was recently completed to investigate whether the consumption of phytochemicals present in a beverage containing powder derived from cranberries (Ocean Spray, Inc., Middleboro, MA) modifies immune function and reduces the severity of cold and flu symptoms in humans (Nantz *et al*., 2013). Fifty-four healthy men and women (aged 21 to 50 years) were recruited and consumed a 15.5 oz water beverage supplemented with cranberry extract powder (approx. 300 mg PAC/day) or placebo for 10 weeks. During the course of the study, 9 subjects withdrew (4 subjects from the treatment group and 5 subjects from the placebo group) mainly due to the taste of the beverage or unable to return for the final blood test. During the 10-week period, subjects recorded if they experienced any cold and flu symptoms, as well as the occurrence of any intestinal distress, which includes nausea, vomiting, diarrhea, and/or abdominal cramps. In addition, subjects completed a questionnaire that included questions on side effects experienced from the beverage. The side effects reported by 4 individuals (2 individuals from each group) were red mouth and fingers, headache and stuffy nose, frequent urination, and hyperactivity. No other safety-related parameters were evaluated.

A randomized double-blind, placebo-controlled clinical trial was conducted to determine the effect of a cranberry beverage on lipids in healthy adults with low to moderate risk for cardiovascular diseases according to the ATP-III criteria (KGK Synergize Inc., 2011). For a period of 12 weeks, 140 subjects (mean age of 48 years) consumed two 15.2 oz beverages [either cranberry beverage or placebo (Ocean Spray Cranberries, U.S.)] daily, for a total PAC intake of approximately 600 mg/day. No statistically significant differences were reported between the test and control groups on levels of low-density lipoprotein cholesterol, high-density lipoprotein (HDL) cholesterol, total cholesterol, total cholesterol/HDL cholesterol ratio, and triglycerides. During the study period, a total of 106 adverse events (mainly related to the gastrointestinal tract) were reported, of which 53 events occurred in 32 subjects from the placebo group and 53 events occurred in 35 subjects from the test group. The authors reported no serious adverse events

or deaths occurred during the study period and no significant differences between groups with respect to the frequency or the number of subjects reporting an adverse event. In addition, there were no significant differences in liver function markers (*i.e.*, alanine transaminase, aspartate transaminase, and gamma-glutamyl transferase), kidney function parameters (estimated glomerular filtration rate), hematology parameters, clinical chemistry parameters, or vital sign measurements (*i.e.*, heart rate, respiratory rate, and body temperature) between groups after 12 weeks of supplementation with either beverage. Based on the conditions of this study, the authors suggested that the cranberry beverage is well tolerated for a period of 12 weeks at levels up to 600 mg of PACs/day.

Chew *et al.* (2018) recently evaluated the health benefits of a beverage containing cranberry extract powder in overweight or obese adults in a randomized, double-blind, placebo-controlled, parallel design trial. Seventy eight men and women (30–70 years of age; BMI 27–35 kg/m^2) with abdominal adiposity (waist: hip > 0.8 for women and > 0.9 for men; waist: height ≥ 0.5) consumed 450 mL placebo or low calorie, high polyphenol cranberry extract beverage (containing 281 mg PAC) daily for 8 weeks. Glucoregulation, as measured by the oral glucose tolerance test, was improved following consumption of cranberry extract powder. High density lipoprotein (HDL) cholesterol, redox status, and vasodilation were increased. C-reactive protein, a biomarker of inflammation, was downregulated and serum insulin levels were reduced compared to placebo.

Due to the use of cranberry preparations as an adjunctive treatment for urinary tract infections, the effects of cranberry juice and extract consumption has been widely examined in human studies (Jepson *et al*., 2012). However, few studies have been conducted to examine parameters relating to the safety of cranberry extract consumption. A study was identified in which women with a history of chronic urinary tract infections consumed 500 mg/day of a cranberry extract or 100 mg trimethoprim for a period of 6 months (McMurdo *et al.*, 2009). The cranberry extract was reported to be well tolerated with fewer individuals in the cranberry treatment group withdrawing from the study based on adverse events experienced while the incidence of adverse events experienced was not statistically significantly different between the cranberry and trimethoprim treatment groups. Valentova *et al*. (2007) identified no safety concerns or biologically significant changes in urinary parameters (most notably acidity) in 65 healthy women consuming 400 or 1,200 mg dried cranberry juice/day for a period of 8 weeks.

Jepson *et al*. (2012) conducted a meta-analysis of randomized, controlled, clinical trials to assess the efficacy of cranberry products in the treatment and prevention of urinary tract infections (UTIs). The authors identified 10 trials, in which a total of 1,049 subjects were enrolled, meeting appropriate quality guidelines. In these trials the subjects consumed a variety of cranberry formulations including juices (30 to 750 mL/day), concentrates (2,000 to 7,500 mg/day), and tablets (400 mg/day). Jepson *et al*. (2012) reported that withdrawal from these studies was primarily related to complaints associated with the daily required consumption of cranberry juice. These specifically related to the taste and the cost of cranberry juice. Side effects reported by study participants were minor and included acid reflux, mild nausea, gastrointestinal upset, and frequency of bowel movements (in the tablet group).

Review and analysis of the published literature revealed that ingestion of large volumes of cranberry juice destabilize warfarin therapy. One case report involved drinking nearly 2 L/day of cranberry juice to prevent recurrent urinary tract infection prior to surgery (Grant, 2004). Another case report was identified in which an individual displaying increased anticoagulation reported consuming 24 oz/day (3 servings/day) of cranberry juice as a source of vitamin C for 2 weeks prior to treatment (Aston *et al*., 2006; Paeng *et al*., 2007). On the basis of these case reports, Pham and Pham (2007) conducted a review of the literature to assess the potential for interaction between cranberry juice and warfarin. The authors identified 3 peer-reviewed case reports and 2 peer-reviewed, prospective, randomized, placebo-controlled clinical trials using

metabolic surrogates of warfarin (flurbiprofen and cyclosporine) in the literature. Following a review of the case reports, Pham and Pham (2007) concluded that 2 of the case reports failed to identify cranberry juice as the sole cause of international normalized ratio (INR) elevation while the third appeared to show a correlation between the effects of cranberry juice and warfarin metabolism. Neither of the clinical trials reviewed established an interaction between cranberry juice and CYP isoenzymes 2C9 and 3A, both of which are necessary in warfarin metabolism. The authors concluded that the available data did not support a clinically relevant interaction between cranberry juice and warfarin. The authors did advise that individuals receiving warfarin therapy should be cautioned about the potential interaction with cranberry juice.

Since the publication by Pham and Pham (2007) 2 additional clinical trials have been conducted in which the interaction between cranberry juice and warfarin was examined. The association between warfarin anticoagulation by cranberry juice was examined in a randomized, controlled trial conducted by Ansell *et al*. (2009). In this trial, 30 patients receiving stable warfarin anticoagulation therapy, (stable defined as an INR ranging between 1.7 and 3.3), consumed either 240 mL of cranberry juice (n=14) or 240 mL of placebo beverage (n=16), matched for color and taste, once daily for a period of 2 weeks. The INR values and plasma levels of R- and S-warfarin were measured during the 2-week period and a 1-week follow-up period. The mean plasma R- and S-warfarin concentrations within the cranberry juice and placebo groups did not differ significantly. Eight participants, 4 from the cranberry juice group and 4 from the placebo group, developed minimally elevated INR ranging between 3.38 and 4.52 during the treatment period. Mean INR differed significantly only on treatment Day 12; while at all other time points no significant differences were observed between the groups. Cranberry juice consumption was reported to have no effect on plasma S- or R-warfarin plasma levels, excluding a pharmacokinetic interaction. The authors concluded that increased anticoagulation observed in individuals concurrently consuming warfarin and cranberry juice may represent a chance temporal association, and that the results observed on Treatment Day 12 were not clinically relevant.

Similarly, the consumption of 600 mL cranberry juice/day was reported to have no impact on the anticoagulant effect of warfarin in 10 healthy volunteers (Lilja *et al*., 2007). In this study, the subject consumed cranberry juice or water for a period of 10 days. On the fifth day the volunteers were administered 10 mg racemic R-S-warfarin, 1 mg tizanidine, and 0.5 mg midazolam, with juice or water, and the plasma warfarin concentrations and thromboplastin time were measured. Consumption of cranberry juice was not associated with a significant change in the peak plasma concentration or area under concentration-time curve (AUC) of the probe drugs or their metabolites as compared to water. A slight decrease in the AUC S warfarin was observed in the individuals consuming cranberry juice.

Ledda *et al.* (2015, 2016) evaluated the preventative effects of a cranberry extract (Anthocran®) on recurrent lower-urinary tract infections (R-UTIs) in various population groups. In the initial study, 22 subjects with a history of R-UTI consumed 1 capsule of Anthocran® daily for 2 months. Both the frequency of UTI episodes and mean duration of the episodes was significantly reduced relative to control. No adverse events were observed (Ledda *et al.,* 2015).

In a second study, Ledda *et al.* (2016) compared the effects of Anthocran® (1 capsule daily for 60 days) to those of placebo on the urinary tract health of elderly men (n=43). The mean number of UTI episodes during supplementation (as compared to the 2 months prior to treatment) was significantly reduced in subjects consuming Anthocran®. No such effect was seen in individuals in the placebo group. No adverse events were observed.

Foxman *et al*. (2015) evaluated the effect of cranberry capsule on the risk of urinary tract infection in a randomized, double-blind, placebo-controlled trial. One hundred sixty female subjects undergoing elective benign gynecological surgery involving urinary catheterization were randomized and received 2 cranberry juice capsules 2 times a day, equivalent to two 8 oz servings of cranberry juice, for 6 weeks after surgery or matching placebo. UTI occurrence was significantly lower in the cranberry treatment group compared with the placebo group. This significant effect remained after adjustment for known confounders (*i.e.* age, UTI history, the frequency of intermittent self-catheterization). There were no treatment differences in the incidence of adverse events. A total of 328 adverse events were reported by 75 participants in the cranberry group, compared to 423 adverse events among 78 participants in the placebo group. Four participants experienced 5 severe adverse events in the cranberry group and 4 participants experienced 7 adverse events in the placebo group. Gastrointestinal upset was the most commonly reported event in both groups [cranberry: n=45 (56%); placebo: n=49 (61%)].

6.3.1.3 Summary

Overall, information from clinical trials with cranberry extract powder and related cranberry products support the safety of Ocean Spray's ingredient under the proposed conditions of use in food. Clinical studies of Ocean Spray's cranberry extract powder demonstrate that the ingredient is well tolerated when consumed at levels up to 600 mg of PACs/day for 12 weeks. The incidence of adverse effects in these studies were comparable to those seen in placebo, and consisted mainly of gastrointestinal complaints, skin discoloration, headache, stuffy nose, frequent urination, and hyperactivity. Similarly, in studies of cranberry products in the treatment and prevention of urinary tract infections in the scientific literature, only minor gastrointestinal side effects were reported.

6.3.1.3.1 Safety in Infants and Children

Ocean Spray's cranberry extract powder is not intended to be marketed to infants, toddlers and children. However, because infants and young children were identified as having the highest mean and 90^{th} percentile consumer-only intakes of any population group (20.0 and 35.2 mg/kg body weight/day, respectively), particular consideration of these potential exposure levels was considered.

Thirteen intervention studies were identified; these publications are summarized in Table 6.3.1.3.1-1 below. Of the 13 intervention studies, 12 were conducted in children with various uropathies predisposing the subject to UTIs [including neuropathic/neurogenic bladder, R-UTI, vesicoureteral reflux (VUR), or urogenital malformations] (Foda *et al.*, 1995; Schlager *et al.*, 1999; Ferrara *et al.*, 2009; Nishizaki *et al.*, 2009; Goj *et al.*, 2010; Maringhini *et al.*, 2010; Afshar *et al.*, 2012; Mutlu and Ekinci, 2012; Salo *et al.*, 2012; Uberos *et al.*, 2012; Dotis *et al.*, 2014; Ledda *et al.*, 2017). The primary objective of these studies was to evaluate the effects of supplementation with cranberry products (*i.e.,* juice, syrup, or capsules) on the incidence or recurrence of UTI. The remaining intervention study was conducted in healthy children, and was designed to evaluate the effects of cranberry juice on nasal and fecal bacterial flora (Kontiokari *et al.*, 2005). Children aged 1 month to 18 years at baseline were included in these intervention studies, and were given cranberry products for up to 24 months.

As noted in several systematic/narrative reviews and meta-analyses identified in the literature search (Williams and Craig, 2009; Dessi *et al.*, 2011; Jepson *et al.*, 2012; Wang *et al.*, 2012b; Vasileiou *et al.*, 2013), dose-response relationships (with respect to safety and efficacy) in the published intervention studies on cranberries in children are difficult to establish due to inconsistencies in the doses of active cranberry components consumed. It is generally recognized that PACs are the active component of cranberries that may interfere with the adhesion of *Escherichia coli* to the urinary tract epithelium, thus potentially reducing

the risk of UTI (Jepson *et al.*, 2012). Where available, information provided in the identified publications, and identified through internet searches for product names, was used to approximate the doses of PACs consumed in each study.

The PAC content of the cranberry products given was approximated for 8 of the 12 intervention studies identified (Foda *et al.*, 1995; Schlager *et al.*, 1999; Kontiokari *et al.*, 2005; Goj *et al.*, 2010; Maringhini *et al.*, 2010; Afshar *et al.*, 2012; Salo *et al.*, 2012; Uberos *et al.*, 2012). In these 8 studies, subjects were given cranberry products providing up to approximately 740 mg PAC/kg body weight/day. Over study durations of 3 to 24 months, no remarkable adverse effects were attributable to the cranberry products consumed. Uberos *et al.* (2012) reported 2 cases of gastrointestinal intolerance and 1 case of cutaneous rash among 75 children (1 month to 13 years of age) with recurrent UTIs who were given 0.2 mL cranberry syrup/kg body weight/day (providing approximately 1.44 mg PAC/kg body weight/day). The study authors reported no significant difference in adverse effects between children given cranberry syrup and standard prophylactic antibiotic therapy (1.6 mg trimethoprim/kg body weight/day), and specified that in the 24 children less than 6 months of age at baseline and assigned to receive cranberry syrup, no adverse effects were reported during the entire study period. Kontiokari *et al.* (2005) reported 2 instances of gastrointestinal symptoms in children given cranberry juice providing approximately 715 mg PAC/kg body weight/day for 3 months, although the study authors went on to note that the cranberry juice was "well accepted" by the study subjects and that gastrointestinal symptoms occurred rarely (*i.e.,* in 1% of subjects receiving cranberry juice). Although these 12 studies were not designed specifically to evaluate the safety of PACs, the lack of remarkable compound-related adverse effects, especially in infants less than 6 months of age, supports the safety of cranberry products providing up to 740 mg PAC/kg body weight/day in children.

For the remaining 4 intervention studies, no information regarding the PAC content of the study product was available (Ferrara *et al.*, 2009; Nishizaki *et al.*, 2009; Mutlu and Ekinci, 2012; Dotis *et al.*, 2014). However, as no adverse effects were reported, the results of these 4 studies provide corroborative evidence of the safety of cranberry products in pediatric populations.

Table 6.3.1.3.1-1 Summary of Studies on Cranberry Products Conducted in Children

Reference	Study Design	Study Population	Objectives	Dose and Duration	PAC Dose	Safety-Related Outcomes
Foda *et al.* (1995)	Single-blind, crossover intervention	Children with neuropathic bladder, receiving clean intermittent catheterization (n=21F, 19M) Mean age: 9.35 years (1.4 to 18 years)	To investigate effects of cranberry juice consumption on incidence of UTI	T: **Ocean Spray** cranberry cocktail (30% cranberry concentrate; 15 mL/kg body weight/day, divided into 3 or 4 doses) C: 15 mL water/kg body weight/day, divided into 3 or 4 doses Duration: 6 months/arm	T: 1.8 to 2.55 mg/kg body weight/day C: 0 mg/kg body weight/day	• 17 subjects dropped out during cranberry intervention – none due to adverse effects • No further safety information reported
Schlager *et al.* (1999)	Randomized, double-blind, placebo-controlled, crossover intervention	Children with neurogenic bladder receiving clean intermittent catheterization (n=8F, 7M) Age: 2 to 18 years	To investigate effects of cranberry juice consumption on incidence of UTI and bacteriuria	T: 2 oz cranberry juice concentrate (**Ocean Spray**)/day (equal to 300 mL cranberry juice cocktail) C: 2 oz cranberry jello powder/day Duration: 3 months/arm	T: 36 to 51 mg/day C: 0 mg/day	• No safety-related information reported
Kontiokari *et al.* (2005)	Randomized, double-blind, placebo-controlled, parallel-arm intervention	Healthy children attending day care (n=341; 169F, 172M)[a] Mean age: 4.3 years (1 to 7 years)	To investigate the acceptability of cranberry juice and changes in bacterial flora	T: 5 mL cranberry juice (41 g **Ocean Spray** cranberry concentrate/L)/kg body weight/day (up to 300 mL; n=171) C: 5 mL placebo juice/kg body weight/day (up to 300 mL; n=170) Duration: 3 months	T: 715 mg/kg body weight/day C: 0 mg/kg body weight/day	• 29 subjects (18 in T group and 11 in C group) dropped out: refusal to drink juice (4T, 6C), parents tired of trial (7T, 3C), rash (0T, 1C), GI symptoms (2T, 0C), illness (2T, 1C), unknown (3T, 0C) • Cranberry juice "well accepted" • GI symptoms noted to be rare (occurring in 1% of T group)

Table 6.3.1.3.1-1 Summary of Studies on Cranberry Products Conducted in Children

Reference	Study Design	Study Population	Objectives	Dose and Duration	PAC Dose	Safety-Related Outcomes
Ferrara *et al.* (2009)	Randomized, double-blind, placebo-controlled, parallel-arm intervention	Girls with ≥1 UTI in previous year, with no anatomical or functional uropathy (n=84) Mean age: 7.5 years (3 to 14 years)	To investigate effects of cranberry juice consumption on incidence of UTI	T1: 50 mL concentrated cranberry juice/day (7.5 g cranberry concentrate and 1.7 g lingonberry concentrate; n=28) T2: 100 mL Lactobacillus GG drink (5 days/month; n=27) C: no intervention (n=29) Duration: 6 months	T1: PAC content NR T2: 0 mg/kg body weight/day C: 0 mg/kg body weight/day	• 4 dropouts: 1 from T1, 1 from T2, and 2 from C (all due to poor compliance) • No "negative reactions" reported • Several subjects complained about the taste of the cranberry juice
Nishizaki *et al.* (2009)	Controlled, parallel-arm intervention	Children (18M, 13F) with verified VUR (Grade <5) Mean age: 32.5 ± 19.6 months (T); 18.2 ± 22.9 months (C)	To investigate effects of cranberry juice consumption on incidence of UTI	T: 100 mL 50% concentrated cranberry juice (**Cranberry UR-50, Kikkoman**)/day (n=12) C: 5 to 10 mg cefaclor/kg body weight/day (n=19) Duration: Subjects withdrawn upon development of recurrent UTI; Mean intervention duration: 17.2 ± 7.9 months (3 to 27 months; T), 10.2 ± 3.3 months (5 to 15 months; C)	T: PAC content NR C: 0 mg/kg body weight/day	• Authors noted that "cranberry juice was safe and well tolerated" • 1 subject could not tolerate the taste of the juice • No adverse effects (*e.g.,* GI symptoms) observed in T group
Goj *et al.* (2010) (abstract only)	Uncontrolled intervention	Children with primary VUR and associated urogenital malformations (n=63; 24M, 39F) Mean age: 20 months (1 to 97 months)	To investigate effects of cranberry capsules consumption on incidence of UTI and adverse effects	T: "commercially available standard concentrated cranberry juice" (IVUMIR), 0.5 mL/kg body weight/day Median duration: 24 months	T: 0.215 mg/kg body weight/day	• No subjects suffered adverse effects

Table 6.3.1.3.1-1 Summary of Studies on Cranberry Products Conducted in Children

Reference	Study Design	Study Population	Objectives	Dose and Duration	PAC Dose	Safety-Related Outcomes
Maringhini *et al.* (2010) (abstract only)	Uncontrolled intervention	Children with ≥2 UTI in previous 6 months (n=79; 56F, 23M) Mean age: 5.2 years (0.5 to 17 years)	To investigate effects of cranberry juice consumption on incidence of UTI	T: cranberry juice (IVUMIR) 0.5 mL/kg body weight, twice per day Mean duration: 6.4 months (1 to 21 months)	T: 0.215 mg/kg body weight/day	• No adverse effects reported
Afshar *et al.* (2012)	Randomized, double-blind, placebo-controlled, parallel-arm intervention	Toilet trained children, M/F, ≤18 years of age, with ≥2 UTIs is previous year (n=20/group); no urological abnormalities (other than primary VUR)[b] Median age: 7 (7 to 18 years)	To investigate effects of cranberry juice consumption on incidence of UTI	T: 2 cc **Ocean Spray** cranberry juice (37% PAC)/kg body weight/day (19F, 1M) C: 2 cc juice (no PAC)/kg body weight/day (20F) Duration: 12 months	T: 740 mg/kg body weight/day C: 0 mg/kg body weight/day	• 6 subjects/group withdrew from study (included in analysis) • 1 subject withdrew due to skin rash (group NR) • No further safety-related information provided
Mutlu and Ekinci (2012)	Randomized, placebo-controlled crossover intervention	Children with neurogenic bladder receiving clean intermittent catheterization (n=20; 7M, 13F) Mean age: 7.25 ± 3.49 years (4 to 18 years)	To investigate effects of cranberry capsules consumption on incidence of UTI	T: cranberry extract capsules (**GNC Company**; 1 capsule/day) C: placebo (1 capsule/day) Duration: 6 months/arm	T: PAC content NR C: 0 mg/kg body weight/day	• No adverse effects reported during intervention
Salo *et al.* (2012)	Randomized, double-blind, placebo-controlled, parallel-arm intervention	Children (M, F) referred to pediatric hospital department due to verified UTI in previous 2 months (n=263)[c] Mean age: 3.8 ± 2.5 years (T); 4.5 ± 2.9 years (C)	To investigate effects of cranberry juice consumption on recurrence of UTI	T: 5 mL cranberry juice/kg body weight/day (up to 300 mL/day, in 1 or 2 daily doses) (**Ocean Spray**, 41 g cranberry concentrate/L juice; n=129; 115F) C: 5 mL placebo juice/kg body weight/day (up to 300 mL/day, in 1 or 2 daily doses) (n=134; 117F) Duration: 6 months	T: 706.5 mg PAC/kg body weight/day (up to 42.39 g/day) C: 0 mg/kg body weight/day	• 8 subjects excluded due to protocol violation (at entry) • 27 dropouts – 16 from T and 11 from C, mostly due to juice refusal (n=7 and 6, respectively) • Other reasons for dropout NR

Table 6.3.1.3.1-1 Summary of Studies on Cranberry Products Conducted in Children

Reference	Study Design	Study Population	Objectives	Dose and Duration	PAC Dose	Safety-Related Outcomes
Uberos *et al.* (2012)	Randomized, double-blind, placebo-controlled intervention	Children 1 month to 13 years of age (112F, 80M), with ≥2 confirmed UTIs in previous 6 months[d] Mean age: 29.5 months (1 month to 13 years)	To investigate effects of cranberry juice consumption on incidence of UTI	T: 0.2 mL cranberry syrup (Ruei Malmaison; 36 mg PAC/5mL)/kg body weight/day (n=75) C: 0.2 mL color-masked trimethoprim (8 mg/mL)/kg body weight/day (n=117) Duration: 12 months	T: 1.44 mg/kg body weight/day C: 0 mg/kg body weight/day	• "GI intolerance" (5C, 2T) • 3 dropouts from each group • 1 case of cutaneous rash in each group • 24 subjects given cranberry were <6 months of age at baseline – no adverse effects over 12 months
Dotis *et al.* (2014) (abstract only)	Randomized controlled intervention	Children with history of recurrent UTI, with non-severe VUR (n=76; 53F, 23M) Age: 2 to 18 years	To investigate effects of cranberry capsules consumption on incidence of UTI	T: 2 capsules/day (**Mirtygil**, Epsilon Health) (n=38) C: not specified (n=38) Duration: 12 months	T: 18 mg/day C: 0 mg/day	• No adverse effects reported in cranberry group
Ledda *et al.* (2017)	Registry supplement Study	Children with a history of recurrent UTI (n =36, 19F, 17M) Age: 12 to 18 years	To investigate effects of cranberry capsules consumption on incidence of UTI	T: 1 capsule (120 mg of Anthocran® cranberry extract, n=19) plus standard management C: Standard management (n=17)	T: 36 mg/day C: 0 mg/day	• No adverse effects reported

C = control; F = female(s); GI = gastrointestinal; M = male(s); NR = not reported; PAC = proanthocyanins; T = treatment; UTI = urinary tract infection; VUR = vesicoureteral reflux.

[a] Sample size calculations performed; required sample size to detect 15% reduction in common respiratory pathogens = 336 subjects (total).

[b] Sample size calculations performed; required sample size to detect 30% reduction in infection rate over 12 months = 20 subjects/arm.

[c] Sample size calculations performed; required sample size to detect 50% reduction in recurrences of UTI over 12 months = 130 subjects/arm.

[d] Sample size calculations performed; required sample size to detect 20% reduction in risk of recurrence of UTI over 12 months = 109 subjects/arm.

6.3.2 Supporting Evidence of Safety

6.3.2.1 Flavonoids

Flavonoids, including flavonols, flavan-3-ols, anthocyanins, and proanthocyanidins, and organic acids (mainly quinic, malic and citric acids) are the main components of cranberry extract powder. A comprehensive search of the literature bearing on the safety of these compounds was in 2018. Databases that were searched in PubMed, Medline, Toxline, Highwire, the Chemical Carcinogenesis Research Information System, the Hazardous Substances Data Bank, the Developmental and Reproductive Toxicology database, the European Union Inventory of Existing Commercial Chemical substances, the U.S. Environmental Protection Agency, the International Association for Research on Cancer, the Domestic Substances List of Canada, the National Toxicology Program, the National Cancer Institute, the World Health Organization, JECFA, the Food and Agricultural Organization, and the U.S. FDA.

A summary of the constituents of various polyphenol rich-GSE as compared to cranberry extract powder is presented in Table 6.3.2.1-1.

Table 6.3.2.1-1 Comparison of Phenolic Contents of Various Polyphenol Containing Extracts

Constituents %	Cranberry Extract Powder	Polyphenol (GSE, GRN 0000125)	Polyphenols (GSKE, GRN 000125)	ActivinR (GSE, GRN 0000124)	GravinolSuper (GSE)
Proanthocyanidins					
Monomers	4.0				
Dimers	14.0	4.4	3.6	54.0	6.6
Dimer Gallates		0.8	0.4		
Trimers	6.0	1.4	2.2	13.0	5.0
Trimer Gallates		0.5	0.2		
Oligomers & Polymers	30.7	74.6	73.8	7	77.7
Sub-total	**50**	**82**	**80**	**76**	**89**
Phenolics*	**35–38**				
Anthocyanins	**5–8**		**2**		
Minor Flavonoids					
Quercetin	0.2–0.9		0.2		
Quercetin-3-Galactoside					
Quercetin-3-Glucoside					
Myricetin	0.09–0.4		0.01		
Kaempferol			0.02		
Sub-total	4–5		0.3		
Monomeric Flavanols					
Catechin		2.9	2.5		
Epicatechin		4.3	2.8		
Monomer gallate		0.2	0.1		
Epigallocatechin					
Epigallocatechingallate					
Sub-total		7	6	3	7

Table 6.3.2.1-1 Comparison of Phenolic Contents of Various Polyphenol Containing Extracts

Constituents %	Cranberry Extract Powder	Polyphenol (GSE, GRN 0000125)	Polyphenols (GSKE, GRN 000125)	ActivinR (GSE, GRN 0000124)	GravinolSuper (GSE)
Non-flavonoid polyphenols					
Gallic acid		0.95	1.87		
Ellagic acid		0.01	0.02		
Caffeic acid		0.01	0.03		
Chlorogenic acid		0.03	0.07		
Resveratrol		0	0.01		
Other		4.08	4.9		
Sub-total		5.98	6.9		
Total Phenolics	90–93	95	95	80	96
Other constituents					
Standardizing carrier (*e.g.*, maltodextrin)	9–22				
Flow agent (*e.g.*, silicon dioxide)	0.8				
Ash		0.52	0.82		
Insoluble matter		0.57	0.42	0.6	0.8
Fatty acids				2.8	
Polysaccharides					
				10.6	
Amino acids				2.1	
Total (%)	**100**	**100**	**100**	**101**	**100**

GSE = grape seed extract; GSKE = grape skin extract; GRN = GRAS Notice number.
* Including phenolic acids (such as benzoic, chlorogenic, 4-hydroxycinnamic, 3,4-dihydroxybenzoic, vanillic, caffeic), anthocyanidins (such as cyanidin-3-galactoside, cyanidin-3-glucoside, cyanidin-3-arabinoside, peonidin-3-galactoside, peonidin-3-glucoside, peonidin-3-arabinoside), flavanols, and flavan-3-ols (such as quercetin, myricetin and their glycosides).

Polyphenols are the most abundant antioxidants in the human diet. Flavonoids can affect a wide range of functions at the tissue, cellular, and molecular levels. Systematic toxicological testing is generally lacking for most flavonoids. Proanthocyanidins, extracted from grape seeds, are widely used mainly as nutritional supplements and more widely studied than proanthocyanidins from other sources. The literature search revealed 3 peer-reviewed publications on the systematic investigation of the toxicological properties of proanthocyanidins from GSE and grape skin extract (GSKE) (Bentivegna and Whitney, 2002; Yamakoshi *et al.,* 2002; Charradi *et al*., 2018). These studies are summarized below.

6.3.2.1.1 Acute Toxicity

Oral gavage doses of 0 (control), 2, and 4 g/kg body weight of GSE were administered to 3 groups of 5 males and 5 females F344 rats, for 14 days (Yamakoshi *et al.*, 2002). Daily observations for overt signs of toxicity were conducted for 14 days post-treatment. Body weights were also recorded. On Day 14, the animals were sacrificed, and a gross necropsy performed for examination of major organs and tissues. No deaths occurred and there was no evidence of acute oral toxicity at either dosage of 2 or 4 g/kg body weight.

6.3.2.1.2 Subchronic Toxicity

Yamakoshi *et al.* (2002) conducted a 90-day sub-chronic toxicity study in 4-week-old male and female F344/DuCrj rats administered GSE in the diet. GSE was administered in the diet at levels of 0.02, 0.2 and 2% (w/w) to 4 groups of 10 male/female rats for 90 days. Routine clinical observations, body weight, and food and water consumption were measured throughout the study period. Hematology and blood chemistry data were evaluated at the end of the study. Urinalysis was also conducted at the end of the study. All animals were sacrificed at the end of the study, organs and tissues examined for gross pathological changes, and selected organs and tissues collected for histopathological examination. Organ weights were also recorded. Additionally, for animals in the high dose group further histopathological examination of the thoracic aorta, trachea, tongue, esophagus, small and large intestines, pancreas, mandibular and mesenteric lymph nodes, mammary and Harderian glands, spinal cord, sciatic and optic nerves, skin, eyes, sternum, femur, and skeletal muscles was conducted.

There were no noticeable signs of toxicity in any of the parameters measured. No clinical abnormalities related to the feeding of the GSE were observed. The food and water intake of treatment and control groups did not differ significantly and as a result the mean body weights of the animals in each group did not differ significantly at any time point. There were no reported significant differences between the blood chemistry, hematology, leukocyte differentials, or urinalysis values between the treatment or control groups. Statistically significant changes included increased epididymis weights and decreased thymus weights in males in the mid-dose group (0.2%) relative to controls; decreased thymus/body weight ratio in mid-dose females, and decreased stomach/body weight ratio in low-dose (0.02%) females relative to controls. Gross examinations at necropsy revealed no compound-related changes. Microscopic changes were noted in some organs (not specified) of both males and females of the control and high-dose groups. Histopathological examination did not reveal any compound-related changes, and the authors concluded that GSE did not induce toxicity at any dose level. The no-observed-adverse-effect level (NOAEL) for GSE toxicity was considered to be 2% in the diet, which was equated to 1,410 mg/kg body weight/day in males and 1,501 mg/kg body weight/day in females. Since GSE is composed of 89.3% proanthocyanidins, the proanthocyanidins-equivalent NOAELs would be 1,259 mg/kg body weight/day (males) and 1,340 mg/kg body weight/day (females).

Based on the results of these studies, the authors concluded a lack of toxicity and supported the use of proanthocyanidin-rich extracts from grape seeds for various foods (Yamakoshi *et al.*, 2002).

Bentivegna and Whitney (2002) conducted a 90-day sub-chronic toxicity study in which Meganatural™ brand GSE and GSKE were administered to 100 male and 100 female CRI:CD (SD) IGS BR Sprague-Dawley rats. The GSE was composed of approximately 90.5% total phenols while GSKE contained 87.3% total phenols expressed as gallic acid equivalents. Catechin, epicatechin, and gallic acid content of the GSE and GSKE test articles were 3.4 and 4.8%, 4.6 and 4.4%, and 2.4 and 1%, respectively. Groups of CD1 (Sprague–Dawley) rats (20 males and 20 females per group) were fed certified rodent diets (PMI Intl., St. Louis, MO) containing GSE at concentrations of 0, 0.63, 1.25, or 2.5% (w/w); GSKE was fed at 2.5% (w/w) only. Rats were individually housed during the study period. Clinical observations were recorded twice daily and body weight and feed consumption measured weekly throughout the study. An ophthalmologic examination was performed on all animals pretest and at the termination of dosing. After 1 month, blood was obtained from 10 rats/sex/group by retrobulbar puncture for interim measurement of clinical pathology. At the end of the study the rats were subjected to a full necropsy, aortic blood samples were collected for hematology, coagulation, and clinical chemistry analyses, selected organs were weighed and a complete list of tissues was preserved from all animals. Histologic examination was performed on all tissues from control and high-dose GSE and GSKE groups.

There were no treatment-related changes that were considered to be of toxicological significance. Increased food consumption was observed in both high-dose groups; however, this was considered by authors to be a compensatory response to the high dietary concentration of the test articles. No biologically or toxicologically significant changes were noted in hematological or coagulation parameters measured after 30 and 97 days, compared to baseline values, and all values were within physiologically normal ranges. Significant decreases in relative and absolute heart weights were observed in the female rats administered the 1.25% GSE and 2.50% GSKE diets, respectively; however, due to a lack of a dose-response relationship, the fact that this was only observed in female rats and that no irregularities were noted in the microscopic examination of the tissue, these findings were not considered by the authors to be treatment-related. A dietary concentration of 2.5% GSE or 2.5% GSKE was considered to be the NOAEL. This was equivalent to a time-weighted average dose over the course of the study of approximately 1.78 g/kg body weight/day GSE or GSKE in male rats and 2.15 g/kg body weight/day in female rats.

The safety of Oligonol®[1], and a grape seed polyphenol (GSP) extract was examined in a toxicity study that included both a 4-week and a 6-month treatment period (Fujii *et al.*, 2007). The toxicity study was conducted in 6- and 7-week-old JIA:ddy male mice consuming either a control diet or diets providing 200 mg/kg body weight/day of a GSP or 3.33, 26.6, or 200 mg/kg body weight/day of Oligonol®. Groups of 6 animals were administered each diet for the 4-week toxicity study and a further 6 animals consumed each diet for 6 months. Oligonol® was reported to contain between 15% and 20% monomers and the monomeric fraction of the GSP was not reported. During the 4-week arm of the study, body weight and food consumption were measured every 2 days and at the end of the experimental period all animals were sacrificed by cervical dislocation. Serum and organs were examined for markers of kidney and liver function and hematological biomarkers. In the 6-month study, the mice were observed daily for mortality and signs of toxicity, or morbidity. Body weight was measured weekly and food consumption was measured every 2 days. At the end of the study period, mice were sacrificed and blood and organs were collected. Serum biochemical indices of liver and kidney function were evaluated. The authors reported that the consumption of diets containing Oligonol® or GSP did not produce any adverse effects at any dose level. As a result, a NOAEL of 200 mg/kg body weight per day, the highest dose tested, was established for Oligonol®. Based on the monomeric content of Oligonol® this corresponds to 40 mg/kg body weight per day. A NOAEL of 200 mg/kg body weight/day, the only dose tested, can also be derived for exposure to GSP. The safety of Oligonol® also was investigated in a 90-day sub-chronic study conducted in male and female Sprague-Dawley rats (Fujii *et al.*, 2008). No significant adverse effects in food consumption, body weight, mortality, clinical chemistry, hematology, gross pathology, and histopathology was reported following gavage administration to rats at doses up to 1,000 mg/kg body weight/day of Oligonol®.

Wister rats (n=6/group) were orally administered 0, 0.4, 4, 8, or 16 g/kg grape seed powder per day in their diets for 2 months (Charradi *et al*., 2018). At the end of the study period rats were euthanized and the brain, hippocampus, heart, liver, kidneys, and adipose tissue were dissected, analyzed, and weighed. Blood and plasma samples were also taken to measure the activity of c-reactive protein, transaminases, creatinemia, uremia, uricemia, lactate dehydrogenase, interleukin-6, interleukin-10, interleukin-17A, xanthine oxidase, fumarase, α-ketoglutarate dehydrogenase, and nicotinamide adenine dinucleotide (NADH) dehydrogenase. Administration of 16 g/kg grape seed powder had no effect on food and energy intake, but there was a reported dose-dependent decrease in body weight. Histopathological examination of the heart and kidney revealed dilation of arterioles, and examination of the brain revealed a size increase of the hippocampal dentate gyrus following 16 g/kg grape seed powder administration. This same dose provided anti-oxidative effects on the brain, heart, liver, and kidney, and in terms of organ weights there

[1] Oligonol® is a manufactured phenolic ingredient containing catechin-type monomers and lower oligomers of proanthocyanidin that are derived *via* the oligomerization of polyphenols, typically proanthocyanidin derived from a variety of fruits (lychees, grapes, apples, persimmons, *etc*).

was a decrease in adipose weight and a slight increase in the relative weight of the hippocampus. No effects on the relative weights of the other organs were reported.

An increase in platelets was seen at the 4 g/kg dose level. The highest dose of grape seed powder caused a slight decrease in plasma transaminases, creatine, urea, xanthine oxidase, alanine aminotransferase , and aspartate aminotransferase. Similarly, 16 g/kg grape seed powder exerted anti-inflammatory properties through an increase in interleukin-10 and adiponectin, and a decrease in interleukin-17A and c-reactive protein. Grape seed protein had no effect on the following intracellular mediators: free iron, calcium, and hydrogen peroxide. The author reported that administration of up to 16 g/kg grape seed extract was safe under the conditions of this study (Charradi *et al*., 2018).

6.3.2.2 Summary

Overall, the published toxicity studies of other proanthocyanidin-rich ingredients (GSE and GSKE) support the safety of Ocean Spray's cranberry extract powder under the proposed conditions of use. The proanthocyanidin content of these ingredients is higher than that in cranberry extract powder (80% vs approximately 50%). The NOAELs in 90-day sub-chronic toxicity evaluations of these ingredients ranged from 1,250 to 2,150 mg/kg body weight/day. These correspond to intakes of 1,000 to 1,720 mg PAC/kg body weight/day, over 100-fold higher than the 6.9 mg PAC/kg body weight/day intake estimated among 90th percentile consumers based on the proposed uses of cranberry extract powder. Likewise, no mutagenic or genotoxic effects have been seen in either *in vitro* or *in vivo* evaluations of these ingredients.

6.3.2.2.1 Mutagenicity and Genotoxicity

Yamakoshi *et al.* (2002) examined the mutagenic potential of a proanthocyanidin-rich GSE in several *in vitro* assays. The *in vitro* assay conducted included an Ames assay conducted in *S. typhimurium* strains TA98 and TA100, which were exposed to doses of 19 to 1,250 µg/plate, and in strains TA1535 and TA1537, which were exposed to doses of 156 to 5,000 µg/plate based on the results of the dose-ranging study. All exposures were conducted in the absence or presence of metabolic activation provided by an S9 mixture. The vehicle was employed as the negative control and benzo[a]pyrene (BaP), AF2, 2AA, NaN_3, and 9-aminoacridine hydrochloride monohydrate (9AA) were employed as positive controls. No significant increase in the number of revertant colonies was observed in any of the test strains exposed to GSE, either in the presence or absence of S9 mix.

The *in vitro* assays conducted by Yamakoshi *et al.* (2002) also included a chromosomal aberration assay conducted in Chinese hamster lung (CHL) cells. In this assay, the cells were exposed continuously to 9.4, 18.8, and 37.5 µg/mL GSE for 24 and 48 hours in the absence of the S9 mixture or continuously exposed to 18.8, 37.5, 75.0, 150.0, and 300.0 µg/mL GSE for 6 hours in the absence and presence of the S9 mixture. An additional group of CHL cells also were exposed also to GSE at doses of 18.8, 37.5, and 75.0 µg/mL in the absence of S9 mix, and then washed and cultured for an additional 18 hours in control media. Individual procyanidin dimers, trimers, and tetramers, isolated from the GSE also were included in this assay and accordingly, CHL cells were exposed to doses of these compounds ranging between 9.4 and 5,000 µg/mL in the absence and presence of the S9 mixture under the same 4 protocols employed for the GSE exposure. Mitomycin C (MMC) and dimethylnitrosamine (DMN) were employed as positive controls for the cells treated in the absence and presence of S9 mix, respectively. No significant increases in the frequency of metaphases with aberrant chromosomes and no aneuploidy or polyploidy was observed in any of the CHL cells cultured with GSE, regardless of the duration of exposure and the presence or absence of S9 mix. No aneuploidy or polyploidy was observed in the CHL cells exposed to procyanidin dimers, trimers, and tetramers in both the activated and non-activated system. The procyanidin dimers and tetramers were

reported to produce weak positive results by increasing the frequency of chromosomal aberrations at high dose levels. MMC and DMN were both observed to induce strong chromosomal aberrations and elevated the frequency of the aberrations.

Yamakoshi *et al.* (2002) also conducted an *in vivo* micronucleus assay in 8-week-old male ddY mice exposed to proanthocyanidin-rich GSEs. The mice were orally administered 2 doses of GSE dissolved in distilled water 24 hours apart with each dose providing 0 (control), 500, 1,000, or 2,000 mg/kg body weight. Peripheral blood was collected from the mice by piercing the ventral tail a further 24 hours after the final dose was administered. The blood was examined and the frequency of micronucleated peripheral reticulocytes (MNRETs) was recorded based on an examination of 1,000 reticulocytes/mouse. MMC was administered under the same dosing regimen as a positive control. No significant difference was observed in the frequency of MNRETs in the samples collected from the control mice and those collected from the treated mice. In summary, Yamakoshi *et al.* (2002) concluded that there was no evidence of mutagenicity based on the Ames test, *in vitro* chromosomal aberration test with CHL cells, and mouse micronucleus test.

6.3.2.2.2 Human Studies

In a human supplementation study, 30 health volunteers (aged 23 to 62 years; 5/sex/group) received either 200 mg grape seed polyphenol, 100 mg Oligonol® (15 to 20% monomers of proanthocyanidins), or 200 mg Oligonol®, daily for 92 days (Fujii *et al.*, 2007). On Days 0, 28, and 92, blood and urine were collected for hematology and renal function analysis. No significant differences were observed in any of the parameters tested, compared to Day 0 and were within normal range. The authors concluded that a dose of 200 mg Oligonol®/day is safe for human consumption.

The safety and tolerability of a grape seed extract rich in proanthocyanidin was evaluated in a clinical trial. Healthy volunteers (n=29) orally consumed 1,000, 1,500, or 2,500 mg of grapeseed extract daily for 4 weeks. In general, no subjects experienced significant adverse events from the consumption of grape seed extract during the study. Two subjects in the highest dose group were reported to have a decrease (although still within normal physiological range) in iron serum at Week 2 when compared to baseline. When measured 2 weeks after the study concluded, these iron serum values returned to near baseline. It was concluded that consumption of 2,500 mg of grape seed extract a day for 4-weeks was generally safe and well tolerated in adults (Sano, 2017).

6.3.2.2.3 Adverse Effects

Various adverse effects have been observed with different flavonoids, including anti-nutritional effects, thyroid toxicity, drug interaction, genotoxicity/carcinogenicity and developmental effects. The following have been reviewed and summarized in the published literature: Very high concentrations of flavonoids in the diet (*e.g.,* myricetin, quercetin, and epigallocatechin gallate) have been postulated to have anti-nutritional effects, including inhibition of proteolysis within the gut, reduced glucose uptake, impaired food utilization, and impaired mineral absorption (Kuo *et al.,* 1998; Hurrell *et al.,* 1999; Kao *et al.,* 2000; Kobayashi *et al.,* 2000; Sayama *et al.,* 2000; Song *et al.,* 2002; Temme and van Hoydonck 2002; Jung *et al.,* 2004; Nelson and Poulter 2004; Johnston *et al.,* 2005). It has been suggested that a high consumption of flavonoids may increase the risk of iron depletion in a population with marginal iron status (Erdman *et al.*, 2007). Several flavonoids have been shown to inhibit thyroid peroxidase and interfere with the biosynthesis of thyroid hormone *via* free radical iodination (Doerge and Sheehan, 2002; Ferreira *et al.,* 2002; Schroder-van der Elst *et al.,* 2004).

Phenolic compounds, including quercetin, myricetin, and kaempferol (0 to 2.7 mg/kg) (Bilyk and Sapers, 1986) have been shown to be mutagenic *in vitro* (Bjeldanes and Chang, 1977; Hardigree and Epler, 1978; MacGregor and Jurd, 1978; Meltz and MacGregor, 1981). Similar results have been obtained *in vitro* with other antioxidant compounds (*e.g.,* vitamin C, TBHQ) likely due to their prooxidant activity at unrealistically high *in vitro* concentrations, not attainable *in vivo*. Although positive *in vitro* data could indicate intrinsic genotoxic properties of a compound, appropriate *in vivo* data determine the biological significance of these *in vitro* signals in most cases (U.S. FDA, 2012). Indeed, the *in vitro* mutagenic properties of quercetin have not been observed *in vivo* following oral administration (Caria *et al*., 1995; Taj and Nagarajan, 1996; Utesch *et al.,* 2008). This discrepancy between positive *in vitro* findings *versus* negative *in vivo* results may be attributed to limited *in vivo* absorption of quercetin as the aglycone, extensive intestinal microbial degradation, and post-absorption enzymatic metabolism acting in concert to limit the potential for *in vivo* adverse effects following quercetin consumption.

Quercetin was also reported to be carcinogenic in long term studies with laboratory animals (Pamukcu *et al*., 1980; NTP, 1992. National Toxicology Program (NTP) reported a significant dose-dependent reduction in body weight gains of males and females and an increased severity of chronic nephropathy, hyperplasia, and neoplasia of the renal tubular epithelium (causing primarily benign tumors of the renal tubular epithelium) in male, but not female rats exposed to 40,000 ppm (mean dose level approximately 2,200 mg/kg body weight/day) quercetin in the diet for 2 years. On this basis, the NTP concluded that, under the conditions of the study, there was some evidence of carcinogenic activity of quercetin in male F344/N rats based on an increased incidence of renal tubule cell adenomas, but no evidence of carcinogenic activity of quercetin in female F344/N rats (NTP, 1992). However, following re-evaluation of the kidney lesions, Hard *et al.* (2007) concluded that the alterations observed in the renal tissues of male rats in this study were the result of a secondary mechanism for renal tumor development (*i.e.,* exacerbation of late-stage progressive chronic nephropathy), with no relevance for extrapolation to humans. In the absence of any differences in the survival rates between quercetin-treated and control animals, morphological abnormalities, or organ-based toxicity, the body weight differences observed between high-dose test and control rats were not considered to be adverse effects. Based on the results of the NTP study, no biologically significant adverse effects are expected at dose levels at or below 2,200 mg quercetin/kg body weight/day.

In addition, as reviewed in GRN 341 (U.S. FDA, 2010), other long-term toxicity and carcinogenicity studies conducted not only in rats, but also in hamsters and mice, have demonstrated the absence of significant histopathological effects, including those of the kidneys, at comparable and in some cases higher dose levels (up to 12 g/kg body weight/day). Overall, the weight of evidence from multiple long-term animal studies, indicates that quercetin has not been shown to be carcinogenic to the kidneys or any other organs/tissues when administered chronically in the diets of laboratory animals.

Although there have been reports of abnormal cells in cell culture and/or reproductive effects following intraperitoneal injection of quercetin, these data are not considered relevant to consumption of quercetin. In the *in vitro* or intraperitoneal studies, quercetin is not metabolized by intestinal microflora prior to its contact with germ cells, which may account for the observed effects. In contrast, oral administration of quercetin during which metabolism by intestinal microflora and first-pass metabolism do occur; sperm abnormalities, developmental toxicity and effects on reproduction endpoints have not been observed (Nandan and Rao, 1983; Stoewsand *et al*., 1984).

The FDA responded with no questions in response to Quercegen Pharma LLC's conclusion that quercetin is GRAS for use in beverages and beverage bases, grain products and pastas, processed fruits and fruit juices, and soft candies at levels up to 500 mg per serving (U.S. FDA, 2010).

6.3.2.3 Phenolic Acids

Chlorogenic acid is one of the most abundant polyphenols in the diet (Gonthier *et al.*, 2003). The daily intake of chlorogenic acid has been reported to be as high as 1,000 mg, based on consumption of 1 L per day of unfiltered coffee (Olthof *et al*., 2001a,b). Caffeic acid, like chlorogenic acid, is a dihydroxycinnamic acid, which is present naturally in coffee and other foods including fruits and vegetables (Hagiwara *et al.,* 1991; Moridani *et al.,* 2001).

The metabolism of chlorogenic acid has been investigated in healthy human subjects and in patients without an intact colon (Olthof *et al.,* 2003). Study participants were provided oral doses of chlorogenic acid, after which their urine was analyzed for chlorogenic acid and its metabolites. In the healthy subjects, approximately half of the ingested dose of chlorogenic acid was recovered in the urine as hippuric acid. In the participants lacking a colon, no hippuric acid and only traces of phenolic acid metabolites were recovered following chlorogenic acid consumption. From these results, the authors concluded that colonic microflora are responsible for creating chlorogenic metabolites, which then enter the systemic circulation (Olthof *et al.,* 2003).

6.3.2.3.1 Acute Toxicity

An oral median lethal dose (LD_{50}) of >1,000 mg/kg body weight was reported for rodents in an acute toxicity study of chlorogenic acid. No deaths were reported to occur following the administration of chlorogenic acid doses ranging from 400 to 2,437 mg/kg body weight (Chaube and Swinyard, 1976; Deng *et al.,* 2000).

6.3.2.3.2 Subchronic Toxicity

In studies conducted in mice and rats from 3 to 10 weeks in duration, NOAELs ranging from 2 to 20 mg of chlorogenic acid/kg diet [providing approximately 165 to 2,000 mg/kg body weight (U.S. FDA, 1993)] were reported following dietary administration of chlorogenic acid (Eklund, 1975; Hirose *et al.,* 1987; Kitts and Wijewickreme, 1994; Frank *et al.,* 2003).

In Balb/c mice, the administration of diets containing 2,000 ppm chlorogenic acid to Balb/c mice, [providing approximately 300 mg/kg body weight/day (U.S. FDA, 1993)], for 10 weeks was reported to have no effect on body, liver, or intestinal weights (Kitts and Wijewickreme, 1994). Exposure to chlorogenic acid significantly reduced several enzyme levels in the intestines of mice; however, no effects were seen in the liver.

In Sprague-Dawley rats, exposure to a diet containing 10,000 ppm of chlorogenic acid, [providing approximately 1,000 mg/kg body weight/day (U.S. FDA, 1993)], for 3 weeks was reported to have no effects on most organ weights, hematological parameters, or food and nutrient metabolism (Eklund, 1975). The weight of the kidney and the adrenal glands was significantly reduced following the exposure of the rats to chlorogenic acid (Eklund, 1975). Similarly, dietary administration of chlorogenic acid to groups of 8 Sprague-Dawley rats for 4 weeks did not affect body weight gain, feed intake, or liver or lung weights (Frank *et al.,* 2003). In this study the animals consumed diets formulated to contain 2 g chlorogenic acid/kg diet, providing daily doses of 350 and 165 mg/kg body weight/day at the beginning and end of study, respectively. The authors reported that chlorogenic acid ingestion was associated with significantly elevated vitamin E levels in lung tissue and increased levels of cholesterol in liver tissues and plasma. No effects on body weight or liver weights were observed in a study in F344 male rats (n=6) in which diets containing 20,000 mg/kg chlorogenic acid [approximately 2,000 mg/kg body weight/day] were administered for 4 weeks (Hirose *et al.,* 1987). No histological changes of the forestomach were observed with the

exception of a mild case of hyperplasia in the central region of the forestomach of 1 of the rats administered chlorogenic acid.

6.3.2.3.3 Developmental and Reproductive Toxicity Studies

The reproductive toxicity potential of chlorogenic acid in pregnant 9-week-old Wistar rats was examined by Chaube and Swinyard (1976). The rats were administered intraperitoneal injections of 0, 5, 40, 60, 100, or 500 mg chlorogenic acid/kg body weight/day throughout Gestational Days 5 to 12. The rats were then sacrificed on Gestational Day 21, and both the mother and fetus were examined for abnormalities. No incidence of maternal or fetal death was reported in the rats administered chlorogenic acid. Approximately 10% of fetuses exposed to chlorogenic acid developed rib defects, while no rib defects appeared in the control group. The only other adverse event reported was 1 fetus exposed to chlorogenic acid (dose not specified) failed to develop a mandible (Chaube and Swinyard, 1976).

In a second developmental toxicity study, pregnant and lactating mice were administered 1% coffee in their diet [approximately 1,500 mg/kg body weight (U.S. FDA, 1993)] and no adverse effects were reported (Stalder *et al.,* 1990).

6.3.2.3.4 Mutagenicity and Genotoxicity

Stich *et al.* (1981) reported that chlorogenic acid is not mutagenic and later studies conducted by the NTP confirmed this result. NTP has confirmed that chlorogenic acid is not mutagenic in 7 strains of *S. typhimurium*; however, chlorogenic acid has been reported to demonstrate both clastogenic and convertogenic properties (Stich *et al.,* 1981). The mutagenic response *in vitro* has been attributed to the generation of hydrogen peroxide, in the presence of transitional metals, as a result of the polyphenolic thermal degradation products of chlorogenic and caffeic acids that reduce oxygen (Schilter *et al.,* 2001). As conditions resulting in the production of hydrogen peroxide including oxygen concentration and iron levels are greater in experimental systems than *in vivo*, the positive findings with chlorogenic acid *in vitro* are of little significance to human health. When irradiated and non-irradiated chlorogenic acid was employed in a micronucleus test in rat bone-marrow cells, negative results were also reported (Hossain *et al.,* 1976). A summary of identified genotoxicity/mutagenicity studies of chlorogenic acid is provided below in Table 6.3.2.3.4-1.

Table 6.3.2.3.4-1 Summary of Identified Genotoxicity/Mutagenicity Studies of Chlorogenic Acid

Test System	Type	Results	Concentration	Reference
Chlorogenic Acid				
Salmonella typhimurium TA98, TA100	Ames Test	Negative	200 µg/mL	Stich *et al.* (1981)
S. typhimurium TA97, TA98, TA100, TA102, TA104, TA1535	Ames Test	Negative (+/-S9)	Up to10,000 µg/plate	NTP (1998)
Saccharomyces cerevisiae	Gene conversion	Positive	200 µg/mL	Stich *et al.* (1981)
Chinese Hamster Ovary Cells	Chromosomal aberrations	Positive	200 µg/mL	Stich *et al.* (1981)
Rat bone-marrow cells	Micronucleus test	Negative	Not specified	Hossain *et al.* (1976)

(+/-S9) = with and without metabolic activation; (+S9) = with metabolic activation; (-S9) = without metabolic activation.

6.3.2.3.5 Carcinogenicity Studies

The carcinogenic potential of chlorogenic acid has been examined in hamsters and mice. In Syrian golden hamsters, the consumption of a diet containing 250 ppm chlorogenic acid for 24 weeks, [providing approximately 30 mg/kg body weight/day (U.S. FDA, 1993)], was reported to induce no tumors in the liver or small intestine (Mori *et al.,* 1986). Swiss albino mice were observed for a year following the direct implantation of cholesterol pellets containing chlorogenic acid into the bladder (Wang *et al.,* 1976). The chlorogenic acid implant did not induce bladder carcinoma within the 1-year observation period (Wang *et al.,* 1976).

To examine the protective potential of chlorogenic acid, Sprague-Dawley rats consumed chlorogenic acid for 7 weeks following their exposure to azoxymethane, a known colonic carcinogen (Exon *et al.,* 1998). The rats consumed 70 mg/kg body weight/day of chlorogenic acid, after which their immune response, formation of azoxymethane-induced crypts, and colonic cell proliferation were examined. Consumption of chlorogenic acid was reported to have no significant effect on any of the parameters examined, indicating that chlorogenic acid did not protect against or enhance azoxymethane carcinogenicity (Exon *et al.,* 1998).

6.3.2.3.6 Human Studies

In a clinical trial in which 20 healthy men consumed a green coffee extract drink containing 140 mg chlorogenic acid daily over a period of 4 months, no significant differences were observed between the test and a placebo group and the drink was reported to be well tolerated (Ochiai *et al.,* 2004). The authors of a randomized, double-blind study designed to compare the effects of normal instant coffee to chlorogenic acid-enriched coffee on the body mass of 30 overweight adults did not report any adverse effects following a 12-week supplementation period (Thom, 2007). In a separate study, the effect of chlorogenic acid on blood plasma homocysteine levels was examined in a clinical trial conducted by Olthof *et al.* (2001b). Although this was not a traditional safety study, the consumption of 2 g chlorogenic acid/day by 10 men and 10 mildly hypertensive women for a period of 1 week was reported not to produce adverse effects (Olthof *et al.,* 2001b). Likewise, no adverse effects or side effects were reported in 2 randomized, double-blind placebo-controlled studies investigating the blood pressure-lowering effects of chlorogenic acid or green coffee bean extract in patients with mild hypertension. In a multicenter study involving 117 mildly hypertensive male volunteers, patients received 0 (placebo), 46, 93, or 185 mg of coffee bean extract once daily for 28 days (Kozuma *et al*., 2005). No adverse effects attributable to the extract occurred. Both systolic and diastolic blood pressures were significantly reduced in those consuming the coffee bean extract compared to the placebo.

A significant decrease in systolic and diastolic blood pressure was observed in 28 mildly hypertensive subjects who consumed 140 mg of chlorogenic acid daily (Watanabe *et al*., 2006a). Furthermore, no serious side effects were experienced by subjects following intake of 299 mg chlorogenic acid in 184 mL of hydroxyhydroquinone (HHQ)-reduced coffee 3 times daily over a period of 4 weeks (Watanabe *et al*., 2006b). Subjects consisted of 12 volunteers with high normal blood pressure or mild hypertension who were not on medication. Systolic and diastolic blood pressures were significantly decreased, and no abnormal changes were observed in pulse rate or routine blood test results. Similar results were observed in a double-blind, randomized controlled trial of 38 high-normotensive and 60 mild hypertensive adult men and women (Chikama *et al*., 2008). In this study, participants ingested 184 mL of a HHQ-containing coffee with a content of 299 mg of chlorogenic acids once daily for 12 weeks or a similar coffee beverage that was reduced in HHQ content. A safety assessment revealed no adverse effects associated with the reduction of HHQ to a level lower than the amounts in commercially available coffee.

6.3.2.4 Summary

Although various flavonoids have been associated with anti-nutritional effects, thyroid toxicity, drug interaction, genotoxicity/carcinogenicity and developmental effects in the literature, these findings are not considered relevant to the safety of cranberry extract powders under the proposed conditions of use in food. These effects were seen at unrealistically high concentrations, occurred *in vitro* assays, or were attributed to mechanisms not considered relevant to extrapolation in humans.

Results of toxicological evaluations of other polyphenols present in cranberry extract powder were reported in the literature. In studies conducted in mice and rats from 3 to 10 weeks in duration, NOAELs ranging from 2 to 20 mg of chlorogenic acid/kg diet, provided approximately 165 to 2,000 mg/kg body weight. Chlorogenic acid had no reproductive, developmental or carcinogenic effects in experimental animals. Furthermore, humans already consume these compounds from other sources in the diet at levels comparable to or above those that would result from the proposed use of cranberry extract powder.

6.4 Allergenicity

Cranberries have a long history of consumption throughout the world and to date no clinical allergenicity concerns regarding the consumption of cranberries have been reported in the scientific literature. Furthermore, since the product is a highly purified polyphenolic substance no allergenicity potential is expected.

6.5 Regulatory Status in other Jurisdictions

6.5.1 Regulatory Status of Cranberry Extract Powder

Ocean Spray's cranberry extract powder was determined to be non-novel in Canada based on a use-level of 80 mg proanthocyanidins per 8 oz serving (proposed for use in beverages). Cranberry juices and dried extracts are also the subject of the Natural and Non-Prescription Health Products Directorate (NHPD) ingredient monographs. In the monograph for cranberry juice, the maximum daily dose recommended for supplement products in Canada is equivalent to 950 mL of cranberry fruit juice, or up to 30 g of fresh cranberries/day, and 400 and 1,200 mg dried extract/day (Health Canada, 2018a,b). Likewise, cranberry extract powder is considered non-novel by Food Standards Australia/New Zealand (FSANZ) when used at a level that ensures that the concentration of phenolic compound is not greater than those in cranberry juice products.

The European Food Safety Authority (EFSA) Panel on Dietetic Products, Nutrition and Allergies (NDA) reviewed a novel food application for cranberry extract powder submitted by Ocean Spray pursuant to Regulation (EC) No 258/97 of the European Parliament and of the Council. Ocean Spray's application proposed addition of cranberry extract powder to fruit-flavored drinks (regular and low calorie), isotonic drinks (including electrolyte types), tea drinks (ready-to-drink, iced), vitamin enhanced waters, yogurts and yogurt drinks with the intention to provide 80 mg PACs per serving. Considering data on the composition, manufacturing process, intake, history of consumption of the source and human data, the Panel had no safety concerns and concluded that the cranberry extract powder is safe as a food ingredient at the proposed uses and use-levels (EFSA, 2017).

6.5.2 Regulatory Status of Related Phenolic Compounds

In the U.S., the regulatory status of other compounds similar to cranberry extract powder in regard to polyphenol composition, have been evaluated. Through the GRAS notification program, the U.S. FDA has provided letters of no objection concerning the use of several ingredients providing phenolic compounds such as anthocyanins, and proanthocyanidins in traditional foods and beverages. GRAS notifications for GSE in 2003 (GRN 000124 and GRN 000125) provide much higher intake estimates for proanthocyanidins (U.S. FDA, 2003a,b). In a GRAS Notification submitted by San Joaquin Valley Concentrates (GRN 000124), San Joaquin estimated the intake of GSE for the U.S. population from the intended use of GSE in beverage and beverage bases, breakfast cereals, fats and oils, frozen dairy desserts and mixes, grain products, milk (whole and skim), milk products, processed fruits and fruit juices to be approximately 150 mg/person/day (3 mg/kg body weight/day) at the mean and 300 mg/person/day (6 mg/kg body weight/day) at the 90th percentile (U.S. FDA, 2003a). San Joaquin described GSE as a complex mixture of polyphenolic compounds, with monomers generally falling into 2 classes: flavonoids and non-flavonoids (U.S. FDA, 2003a). The flavonoid polymers that are known as proanthocyanidins contain a specific flavonoid (flavonols) as monomers. The non-flavonoid polymers are composed of esters of the monomers gallic acid or hexahydroxydiphenyl and a polyol (such as D-glucose). Other non-flavonoid polyphenols are composed of the monomers caffeic acid, chlorogenic acid, and resveratrol. San Joaquin noted that GSE is composed of approximately 74 to 78% proanthocyanidins and <6% free flavanol monomers (U.S. FDA, 2003a). As such, the use of GSE under GRN 000124 provides 111 to 117 mg/day (2.2 to 2.3 mg/kg body weight/day) of proanthocyanidins at the mean and 223 to 234 mg/day (4.4 to 4.7 mg/kg body weight/day) of proanthocyanidins at the 90th percentile (U.S. FDA, 2003a).

In a GRAS notification (GRN 000125), Polyphenolics Inc. estimated the combined intake of GSE and grape pomace extract (GPE) for the U.S. population from their uses in fruit juices, fruit flavored beverages, fruit flavored beverage mixes, and carbonated fruit flavored beverages to be approximately 70 mg/person/day (mean) and 130 mg/person/day (90th percentile) (U.S. FDA, 2003b). Polyphenolics Inc. described GSE and GPE as mixtures of chemicals comprised predominantly of polyphenolic compounds (U.S. FDA, 2003b). The polyphenols are classified according to their monomeric building blocks. For GSE and GPE, these monomers are of 2 classes: flavonoids and non-flavonoids. The flavonoid polymers that are known as proanthocyanidins contain a specific flavonoid (flavonols) as monomers. The non-flavonoid polymers are composed of esters of the monomers gallic acid or hexahydroxydiphenyl and a polyol (such as D-glucose). Other non-flavonoid polyphenols are present in GSE and GPE are composed of the monomers caffeic acid, chlorogenic acid, and resveratrol.

6.6 Expert Panel

Ocean Spray has concluded that its cranberry extract, manufactured consistent with cGMP and meeting food-grade specifications, is GRAS for use as an ingredient in beverages and beverage bases and processed fruits and fruit juices, as described in Part 1.3, on the basis of scientific procedures. Ocean Spray's conclusion on the GRAS status of cranberry extract powder under the conditions of its intended use is based on data generally available in the public domain and the long history of safe consumption of cranberries and cranberry juice, as well as published safety studies on compositionally similar cranberry extracts, related phenolic compounds, and isolated flavonoid components.

A Panel of Experts (the Expert Panel) who are qualified by scientific training and experience to evaluate the safety of food ingredients unanimously concluded on the GRAS status of the cranberry extract powder under conditions of its intended use. The Expert Panel consisted of the following qualified scientific experts: Dr. John Thomas (Adjunct Professor, Indiana University School of Medicine), Dr. Robert Nicolosi (Professor Emeritus, University of Massachusetts Lowell) and Dr Joseph Borzelleca (Professor Emeritus, Virginia Commonwealth University, School of Medicine)[2].

The Expert Panel, convened by Ocean Spray, independently and critically evaluated all data and information presented herein and concluded that cranberry extract powder, meeting appropriate food-grade specifications and manufactured consistent with current Good Manufacturing Practice, is safe and suitable for use as an ingredient in beverages and beverage bases and processed fruits and fruit juices, as described in Part 1.3, and is GRAS based on scientific procedures. A summary of data and information reviewed by the Expert Panel, and evaluation of such data as it pertains to the proposed GRAS uses of the cranberry extract powder, is presented in Appendix 2.

6.7 Conclusion

Ocean Spray intends to market cranberry extract powder in beverages and beverage bases and processed fruits and fruit juices. The main components in cranberry extract powder include polyphenols, specifically proanthocyanidins (~55%), anthocyanins (5 to 8%), phenolics (35 to 38%), and organic acids (0.4 to 0.5%). Cranberry extract powder is produced through a physical extraction of these compounds from cranberry juice concentrate. The other typical components of cranberry juice (*i.e.,* sugars and organic acids) are removed. The phenolic components are not selectively concentrated during the manufacture of cranberry extract powder and remain present at the same ratios as in cranberry juice. The powder is intended to be added into beverages at levels which result in phenolic concentrations similar to those present in cranberry juice cocktail.

Microbiological and other chemical analyses conducted demonstrate that cranberry extract powder conforms to product specifications. Cranberry extract powder is stable for several years when kept under dry conditions at room temperature and below.

Cranberries have a long history of consumption throughout the world and to date no clinical allergenicity concerns regarding the consumption of cranberries have been reported in the scientific literature. The safety of cranberry extract powder is based on the manufacturing process as well as the specifications of production, which are clearly outlined and are performed according to GMP, and is largely supported by a history of use due to the natural presence of polyphenols in food (*i.e*., berries, fruits, and vegetables). The daily background intake of flavonoids, particularly PACs and anthocyanins within these different food categories has been estimated by Scalbert and Williamson (2000) and Santos-Buelga and Scalbert (2000) to be in the range of 460 to 1,000 mg. Based on the high levels of anthocyanins present in fruits and vegetables, intakes of >100 mg/day could be achieved with regular consumption of select fruits or berries, such as blackberries, raspberries, blueberries, or Concord grapes (Wu *et al.*, 2006). Erdman *et al.* (2007) suggests a consumption of 58 mg proanthocyanidins per day for an average American consumer, while Wang *et al*. (2011) estimated proanthocyanidin intake at 95 mg/day.

[2] The panelists participated in their individual capacities. Institutional affiliations are provided for identification purposes only.

Exposure to cranberry extract powder based on the conditions of intended food-use is comparable to or lower than those in other foods. On a consumer-only basis, the resulting mean and 90th percentile intakes of cranberry extract powder from all proposed food-uses, were estimated to be 334 mg/person/day (5.9 mg/kg body weight/day) and 638 mg/person/day (13.0 mg/kg body weight/day), respectively. Mean and 90th percentile intakes of proanthocyanidins from all proposed food-uses of cranberry extract powder were estimated to be 178 mg/person/day (3.1 mg/kg body weight/day) and 340 mg/person/day (6.9 mg/kg body weight/day), respectively. Among the total population (all ages), the mean and 90th percentile intakes of anthocyanins from cranberry extract powder were determined to be 27 and 51 mg/person/day, respectively. Similarly, the mean and 90th percentile intakes of phenolics among the total population (all ages) were determined to be 136 and 259 mg/person/day, respectively. Likewise, the mean and 90th percentile intakes of organic acids from cranberry extract powder among the total population (all ages) were determined to be 2.1 and 4.1 mg/person/day, respectively. These levels and relative ratios of these components are comparable to those resulting from consumption of cranberry juice cocktail.

These intake estimates align with current intakes from consumption of cranberry juice and are well below current dietary exposure to polyphenols. Comparison of the estimated levels of intake to the levels in the diet and the available published data demonstrate that the proposed use of cranberry extract powder in beverages described herein, is not expected to be a safety concern. In addition, existing cranberry juice beverages available in the market contain much higher levels of polyphenols when compared to the level of polyphenols proposed for beverage use from cranberry extract powder. Existing cranberry juice beverages available in the market contain proanthocyanidins (*i.e*., 576 mg per serving), phenolics (*i.e.,* 736 mg per serving), and anthocyanins (*i.e*., 53 mg per serving) at levels much greater than the levels proposed for beverage use from cranberry extract powder.

In addition, cranberry extract powder is expected to be safe for human consumption with no significant health risks based on the safety data on cranberry juice and concentrates, the major phenolic constituents (*i.e*., proanthocyanidins and phenolics), and on extracts similar in phenolic contents to cranberry extract powder. Palikova *et al.* (2010) evaluated the safety of various cranberry extracts at an average dose of up to 0.60 mg/kg body weight/day of phenolic compounds following dietary administration to 10-week-old male Wistar rats for a period of 14 weeks. The authors reported no significant differences in weight gain, organ weights, clinical signs of toxicity, and food consumption between the control and test groups. In addition, the experimental diets did not significantly affect hematological, clinical chemistry, or histopathological examinations. No genotoxicity was seen when these extracts were evaluated in the comet assay. Although this study was not conducted with Ocean Spray's material *per se*, the test materials would have contained very similar types, at least qualitatively, of polyphenolics relative to Ocean Spray's cranberry extract powder. Analysis of PACs in commercial products enables the Ocean Spray's cranberry extract powder to be considered substantially equivalent to those other cranberry extract products currently on the market, and the results of Palikova *et al*. (2010) corroborate the safety of Ocean Spray's product.

As cranberry preparations have been used as an adjunctive treatment for urinary tract infections, the effects of cranberry juice and extract consumption has been widely studied in human studies (Jepson *et al*., 2012). Human clinical trials have demonstrated the safety of Ocean Spray's cranberry extract at doses providing up to 600 mg of PACs per day for up to 12 weeks.

Likewise, a number of human clinical studies had previously been conducted with other cranberry juice cocktails containing 30% concentrates (30 to 300 mL/day), or cranberry capsules (400 to 7,500 mg/day) (Jepson *et al*., 2012). None of these studies have indicated any serious, systemic adverse effects of cranberry consumption. Although the adult general population are the target consumers of cranberry extract product and it is not intended to be marketed to infants, toddlers and children of below 19 years of age, safety in these subpopulations was considered given the potential for high intakes (on a body weight basis) by these consumers. A study conducted in young children of ages 1 month to 1 years who were administered cranberry syrup for a period of 1 year showed no adverse findings following chronic daily administration (Uberos *et al*., 2012). The polyphenol content of the cranberry syrup was determined analytically to be similar to that of the Ocean Spray cranberry extract powder. In addition to the study of Uberos *et al.*, 2012), additional intervention studies with cranberry products in children were identified in the literature. There was no indication of adverse effects of exposure in any study.

The safety of cranberry extract powder is also supported by the safety data available on its constituents (*e.g*., proanthocyanidins, anthocyanins, and other phenolics). The toxicity of proanthocyanidin-rich GSE and grape seed powder has been extensively studied in animals. One acute toxicity study and 4 sub-chronic toxicity studies (two 90-day and one 6-month study) have been performed in mice and rats. A NOAEL was reported up to 1,250 mg/kg body weight/day (males) and 1,340 mg/kg body weight (females) in a 90-day study (Yamakoshi *et al.*, 2002).

The genotoxicity of proanthocyanidin-rich GSE was evaluated in 3 published studies, including an Ames bacterial mutagenicity assay, an *in vitro* chromosome aberration assay in Chinese hamster lung cells, and an *in vivo* mouse micronucleus study. The results from these studies suggest that the consumption of proanthocyanidin-rich GSE would not pose a genotoxic risk. The basis for this conclusion include (a) proanthocyanidin-rich GSE was not mutagenic in the Ames test in the presence or absence of metabolic activation (S9); (b) no chromosomal aberrations were detected in the chromosomal aberration assay in the presence or absence of S9; and (c) no significant differences in the frequency of micronucleated peripheral reticulocytes were reported in the test group when compared to the control group in the *in vivo* rat bone marrow micronucleus test.

Although phenolic compounds, including quercetin, myricetin, and kaempferol present at low levels in cranberry juice extract (at levels comparable to those in cranberry juice cocktail) have been reported to be genotoxic *in vitro*, these results aren't considered to be relevant *in vivo*. Furthermore, the NTP concluded there was some evidence of carcinogenic activity of quercetin in male F344/N rats based on an increased incidence of renal tubule cell adenomas. A re-evaluation of these lesions, (Hard *et al.* 2007) indicates these lesions were the result of a secondary mechanism for renal tumor development (*Le.,* exacerbation of late-stage progressive chronic nephropathy), with no relevance for extrapolation to humans.

Collectively, an analysis of the manufacturing, estimates of consumer exposure, and toxicological data on the polyphenolic constituents of cranberry extract powder supports the conclusion that cranberry extract powder can be safely used under the proposed conditions of use based on scientific procedures.

Part 7. §170.255 List of Supporting Data and Information

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Part	Section §	Section Title
101—Food labeling	101.12	Reference amounts customarily consumed per eating occasion
170—Food additives	170.3	Definitions
	170.30	Eligibility for classification as generally recognized as safe (GRAS).
172—Food additives permitted for direct addition to food for human consumption	172.480	Silicon dioxide

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APPENDIX A
Certificates of Analysis

AVOCA, INC.

PO Box 129
Merry Hill, NC 27957
Phone 252-482-2133 Fax 252-482-8622

CERTIFICATE OF ANALYSIS

Cranberry Extract Powder-Type R

September 15, 2009

LOT NO:	8498-09-02 (P5-9158-1)
% MOISTURE:	3.38%
APPEARANCE:	Fine, free flowing and light-dark red in color
PACs (DMAC):	57.39% dwb
TOTAL PHENOLICS (GAE):	47.89% dwb
SOLUBILITY:	100%, with no visible insoluble particles
RESIDUAL ETHANOL:	<40 ppm wwb
FLOW AGENT SiO2:	0.430%
SCREEN ANAYSIS:	100% through 30 mesh screen

MICROBIOLOGICAL:

TOTAL AEROBIC PLATE COUNT	<10 CFU/g
MOLD	<10 CFU/g
YEAST	<10 CFU/g

[redacted]

Michele L. Phelps
Quality Control Manager
Avoca, Inc.

AVOCA, INC.

PO Box 129
Merry Hill, NC 27957
Phone 252-482-2133 Fax 252-482-8622

CERTIFICATE OF ANALYSIS

Cranberry Extract Powder-Type R

September 15, 2009

LOT NO:	8498-09-03 (P5-9173-1)
% MOISTURE:	4.15%
APPEARANCE:	Fine, free flowing and light-dark red in color
PACs (DMAC):	58.15% dwb
TOTAL PHENOLICS (GAE):	46.14% dwb
SOLUBILITY:	100%, with no visible insoluble particles
RESIDUAL ETHANOL:	<40 ppm wwb
FLOW AGENT SiO2:	0.643%
SCREEN ANAYSIS:	100% through 30 mesh screen

MICROBIOLOGICAL:

TOTAL AEROBIC PLATE COUNT	<10 CFU/g
MOLD	<10 CFU/g
YEAST	<10 CFU/g

[redacted]

Michele L. Phelps
Quality Control Manager
Avoca, Inc.

AVOCA, INC.

PO Box 129
Merry Hill, NC 27957
Phone 252-482-2133 Fax 252-482-8622

CERTIFICATE OF ANALYSIS

Cranberry Extract Powder-Type R

IAN 29299000
Lot Number 8498-09-04-02SD (aka P5-9362-1)
April 20, 2011

Specification	*Value*
% Moisture	3.87%
PACs (%DWB; standardized with maltodextrin)	55.45%
Phenolics (GAE; %DWB)	44.91%
Solubility (1.5gm powder in 1000 ml 50°F water.	100%, with no visible insoluble particles
Ethanol Content (PPM)	<40 ppm
Flow Agent SiO_2 (%)	0.14%
Screen Analysis	100% through 30 mesh screen
Appearance	Fine, free flowing and deep red in color
Flavor & Aroma	Earthy aroma with no burnt character
Microbiological	
Yeast and Mold	<10 CFU/g
Aerobic Plate Count	<10 CFU/g
Coliform	<3 MPN/g
E. coli	<3 MPN/g
Salmonella	Absent/375g

[redacted]
Quality Control Manager
Avoca, Inc.

Cranessence Powders (Results in ppb)

iLab #	Lot #	As	Cd	Pb	Hg
AS-110511-001	**CE 8498-09-02**	75.5	<10	109	<10
AS-110511-002	**CE 8498-09-03**	95	<10	110	<10
AS-110511-003	**CE 8498-09-04**	71.9	<10	42.3	<10

APPENDIX B
Analytical Methods

METHOD 200.8

DETERMINATION OF TRACE ELEMENTS IN WATERS AND WASTES BY INDUCTIVELY COUPLED PLASMA - MASS SPECTROMETRY

Revision 5.4
EMMC Version

S.E. Long (Technology Applications Inc.), T.D. Martin, and E.R. Martin - Method 200.8, Revisions 4.2 and 4.3 (1990)

S.E. Long (Technology Applications Inc.) and T.D. Martin - Method 200.8, Revision 4.4 (1991)

J.T. Creed, C.A. Brockhoff, and T.D. Martin - Method 200.8, Revision 5.4 (1994)

ENVIRONMENTAL MONITORING SYSTEMS LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268
METHOD 200.8

DETERMINATION OF TRACE ELEMENTS IN WATERS AND WASTES BY INDUCTIVELY COUPLED PLASMA - MASS SPECTROMETRY

1.0 SCOPE AND APPLICATION

1.1 This method provides procedures for determination of dissolved elements in ground waters, surface waters and drinking water. It may also be used for determination of total recoverable element concentrations in these waters as well as wastewaters, sludges and soils samples. This method is applicable to the following elements:

Analyte		Chemical Abstract Services Registry Number (CASRN)
Aluminum	(Al)	7429-90-5
Antimony	(Sb)	7440-36-0
Arsenic	(As)	7440-38-2
Barium	(Ba)	7440-39-3
Beryllium	(Be)	7440-41-7
Cadmium	(Cd)	7440-43-9
Chromium	(Cr)	7440-47-3
Cobalt	(Co)	7440-48-4
Copper	(Cu)	7440-50-8
Lead	(Pb)	7439-92-1
Manganese	(Mn)	7439-96-5
Mercury	(Hg)	7439-97-6
Molybdenum	(Mo)	7439-98-7
Nickel	(Ni)	7440-02-0
Selenium	(Se)	7782-49-2
Silver	(Ag)	7440-22-4
Thallium	(Tl)	7440-28-0
Thorium	(Th)	7440-29-1
Uranium	(U)	7440-61-1
Vanadium	(V)	7440-62-2
Zinc	(Zn)	7440-66-6

Estimated instrument detection limits (IDLs) for these elements are listed in Table 1. These are intended as a guide to instrumental limits typical of a system optimized for multielement determinations and employing commercial instrumentation and pneumatic nebulization sample introduction. However, actual method detection limits (MDLs) and linear working ranges will be dependent on the sample matrix, instrumentation and selected operating conditions. Given in Table 7 are typical MDLs for both total recoverable determinations by "direct analysis" and where sample digestion is employed.

1.2 For reference where this method is approved for use in compliance monitoring programs [e.g., Clean Water Act (NPDES) or Safe Drinking Water Act (SDWA)] consult both the appropriate sections of the Code of Federal Regulation (40 CFR Part 136 Table 1B for NPDES, and Part 141 § 141.23 for drinking water), and the latest Federal Register announcements.

1.3 Dissolved elements are determined after suitable filtration and acid preservation. In order to reduce potential interferences, dissolved solids should not exceed 0.2% (w/v) (Section 4.1.4).

1.4 With the exception of silver, where this method is approved for the determination of certain metal and metalloid contaminants in drinking water, samples may be analyzed directly by pneumatic nebulization without acid digestion if the samples have been properly preserved with acid and have turbidity of <1 NTU at the time of analysis. This total recoverable determination procedure is referred to as "direct analysis".

1.5 For the determination of total recoverable analytes in aqueous and solid samples a digestion/extraction is required prior to analysis when the elements are not in solution (e.g., soils, sludges, sediments and aqueous samples that may contain particulate and suspended solids). Aqueous samples containing suspended or particulate material ≥1% (w/v) should be extracted as a solid type sample (Section 11.2.2).

1.6 The total recoverable sample digestion procedure given in this method is not suitable for the determination of volatile organo-mercury compounds. However, for "direct analysis" of drinking water (turbidity <1 NTU), the combined concentrations of inorganic and organo-mercury in solution can be determined by "direct analysis" pneumatic nebulization provided gold is added to both samples and standards alike to eliminate memory interference effects.

1.7 Silver is only slightly soluble in the presence of chloride unless there is a sufficient chloride concentration to form the soluble chloride complex. Therefore, low recoveries of silver may occur in samples, fortified sample matrices and even fortified blanks if determined as a dissolved analyte or by "direct analysis" where the sample has not been processed using the total recoverable mixed acid digestion. For this reason it is recommended that samples be digested prior to the determination of silver. The total recoverable sample digestion procedure given in this method is suitable for the determination of silver in aqueous samples containing concentrations up to 0.1 mg/L. For the analysis of wastewater samples containing higher concentrations of silver, succeeding smaller volume, well mixed sample aliquots must be prepared until the analysis solution contains <0.1 mg/L silver. The extraction of solid samples containing concentrations of silver >50 mg/kg should be treated in a similar manner.

1.8 The total recoverable sample digestion procedure given in this method will solubilize and hold in solution only minimal concentrations of barium in the presence of free sulfate. For the analysis of barium in samples having varying

and unknown concentrations of sulfate, analysis should be completed as soon as possible after sample preparation.

1.9 This method should be used by analysts experienced in the use of inductively coupled plasma mass spectrometry (ICP-MS), the interpretation of spectral and matrix interferences and procedures for their correction. A minimum of six months experience with commercial instrumentation is recommended.

1.10 Users of the method data should state the data-quality objectives prior to analysis. Users of the method must document and have on file the required initial demonstration performance data described in Section 9.2 prior to using the method for analysis.

2.0 SUMMARY OF METHOD

2.1 An aliquot of a well mixed, homogeneous aqueous or solid sample is accurately weighed or measured for sample processing. For total recoverable analysis of a solid or an aqueous sample containing undissolved material, analytes are first solubilized by gentle refluxing with nitric and hydrochloric acids. After cooling, the sample is made up to volume, is mixed and centrifuged or allowed to settle overnight prior to analysis. For the determination of dissolved analytes in a filtered aqueous sample aliquot, or for the "direct analysis" total recoverable determination of analytes in drinking water where sample turbidity is <1 NTU, the sample is made ready for analysis by the appropriate addition of nitric acid, and then diluted to a predetermined volume and mixed before analysis.

2.2 The method describes the multi-element determination of trace elements by ICP-MS.[1-3] Sample material in solution is introduced by pneumatic nebulization into a radiofrequency plasma where energy transfer processes cause desolvation, atomization and ionization. The ions are extracted from the plasma through a differentially pumped vacuum interface and separated on the basis of their mass-to-charge ratio by a quadrupole mass spectrometer having a minimum resolution capability of 1 amu peak width at 5% peak height. The ions transmitted through the quadrupole are detected by an electron multiplier or Faraday detector and the ion information processed by a data handling system. Interferences relating to the technique (Section 4.0) must be recognized and corrected for. Such corrections must include compensation for isobaric elemental interferences and interferences from polyatomic ions derived from the plasma gas, reagents or sample matrix. Instrumental drift as well as suppressions or enhancements of instrument response caused by the sample matrix must be corrected for by the use of internal standards.

3.0 DEFINITIONS

3.1 **Calibration Blank** - A volume of reagent water acidified with the same acid matrix as in the calibration standards. The calibration blank is a zero standard and is used to calibrate the ICP instrument (Section 7.6.1).

3.2 **Calibration Standard (CAL)** - A solution prepared from the dilution of stock standard solutions. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration (Section 7.4).

3.3 **Dissolved Analyte** - The concentration of analyte in an aqueous sample that will pass through a 0.45 µm membrane filter assembly prior to sample acidification (Section 11.1).

3.4 **Field Reagent Blank (FRB)** - An aliquot of reagent water or other blank matrix that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, exposure to the sampling site conditions, storage, preservation, and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment (Section 8.5).

3.5 **Instrument Detection Limit (IDL)** - The concentration equivalent to the analyte signal which is equal to three times the standard deviation of a series of 10 replicate measurements of the calibration blank signal at the selected analytical mass(es). (Table 1).

3.6 **Internal Standard** - Pure analyte(s) added to a sample, extract, or standard solution in known amount(s) and used to measure the relative responses of other method analytes that are components of the same sample or solution. The internal standard must be an analyte that is not a sample component (Sections 7.5 and 9.4.5).

3.7 **Laboratory Duplicates (LD1 and LD2)** - Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicates precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.8 **Laboratory Fortified Blank (LFB)** - An aliquot of LRB to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control and whether the laboratory is capable of making accurate and precise measurements (Sections 7.9 and 9.3.2).

3.9 **Laboratory Fortified Sample Matrix (LFM)** - An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations (Section 9.4).

3.10 **Laboratory Reagent Blank (LRB)** - An aliquot of reagent water or other blank matrices that are treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, and internal standards that are used with other samples. The LRB is used to determine if method analytes or other interferences

are present in the laboratory environment, reagents, or apparatus (Sections 7.6.2 and 9.3.1).

3.11 **Linear Dynamic Range (LDR)** - The concentration range over which the instrument response to an analyte is linear (Section 9.2.2).

3.12 **Method Detection Limit (MDL)** - The minimum concentration of an analyte that can be identified, measured, and reported with 99% confidence that the analyte concentration is greater than zero (Section 9.2.4 and Table 7).

3.13 **Quality Control Sample (QCS)** - A solution of method analytes of known concentrations which is used to fortify an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check either laboratory or instrument performance (Sections 7.8 and 9.2.3).

3.14 **Solid Sample** - For the purpose of this method, a sample taken from material classified as either soil, sediment or sludge.

3.15 **Stock Standard Solution** - A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source (Section 7.3).

3.16 **Total Recoverable Analyte** - The concentration of analyte determined either by "direct analysis" of an unfiltered acid preserved drinking water sample with turbidity of <1 NTU (Section 11.2.1), or by analysis of the solution extract of a solid sample or an unfiltered aqueous sample following digestion by refluxing with hot dilute mineral acid(s) as specified in the method (Sections 11.2 and 11.3).

3.17 **Tuning Solution** - A solution which is used to determine acceptable instrument performance prior to calibration and sample analyses (Section 7.7).

3.18 **Water Sample** - For the purpose of this method, a sample taken from one of the following sources: drinking, surface, ground, storm runoff, industrial or domestic wastewater.

4.0 INTERFERENCES

4.1 Several interference sources may cause inaccuracies in the determination of trace elements by ICP-MS. These are:

4.1.1 Isobaric elemental interferences - Are caused by isotopes of different elements which form singly or doubly charged ions of the same nominal mass-to-charge ratio and which cannot be resolved by the mass spectrometer in use. All elements determined by this method have, at a minimum, one isotope free of isobaric elemental interference. Of the analytical isotopes recommended for use with this method (Table 4), only molybdenum-98 (ruthenium) and selenium-82 (krypton) have isobaric elemental interferences. If alternative analytical isotopes having higher

natural abundance are selected in order to achieve greater sensitivity, an isobaric interference may occur. All data obtained under such conditions must be corrected by measuring the signal from another isotope of the interfering element and subtracting the appropriate signal ratio from the isotope of interest. A record of this correction process should be included with the report of the data. It should be noted that such corrections will only be as accurate as the accuracy of the isotope ratio used in the elemental equation for data calculations. Relevant isotope ratios should be established prior to the application of any corrections.

4.1.2 Abundance sensitivity - Is a property defining the degree to which the wings of a mass peak contribute to adjacent masses. The abundance sensitivity is affected by ion energy and quadrupole operating pressure. Wing overlap interferences may result when a small ion peak is being measured adjacent to a large one. The potential for these interferences should be recognized and the spectrometer resolution adjusted to minimize them.

4.1.3 Isobaric polyatomic ion interferences - Are caused by ions consisting of more than one atom which have the same nominal mass-to-charge ratio as the isotope of interest, and which cannot be resolved by the mass spectrometer in use. These ions are commonly formed in the plasma or interface system from support gases or sample components. Most of the common interferences have been identified[3], and these are listed in Table2 together with the method elements affected. Such interferences must be recognized, and when they cannot be avoided by the selection of alternative analytical isotopes, appropriate corrections must be made to the data. Equations for the correction of data should be established at the time of the analytical run sequence as the polyatomic ion interferences will be highly dependent on the sample matrix and chosen instrument conditions. In particular, the common ^{82}Kr interference that affects the determination of both arsenic and selenium, can be greatly reduced with the use of high purity krypton free argon.

4.1.4 Physical interferences - Are associated with the physical processes which govern the transport of sample into the plasma, sample conversion processes in the plasma, and the transmission of ions through the plasma-mass spectrometer interface. These interferences may result in differences between instrument responses for the sample and the calibration standards. Physical interferences may occur in the transfer of solution to the nebulizer (e.g., viscosity effects), at the point of aerosol formation and transport to the plasma (e.g., surface tension), or during excitation and ionization processes within the plasma itself. High levels of dissolved solids in the sample may contribute deposits of material on the extraction and/or skimmer cones reducing the effective diameter of the orifices and therefore ion transmission. Dissolved solids levels not exceeding 0.2% (w/v) have been recommended[3] to reduce such effects. Internal standardization may be effectively used to compensate for many physical interference effects.[4] Internal standards ideally should have similar

analytical behavior to the elements being determined.

4.1.5 Memory interferences - Result when isotopes of elements in a previous sample contribute to the signals measured in a new sample. Memory effects can result from sample deposition on the sampler and skimmer cones, and from the buildup of sample material in the plasma torch and spray chamber. The site where these effects occur is dependent on the element and can be minimized by flushing the system with a rinse blank between samples (Section 7.6.3). The possibility of memory interferences should be recognized within an analytical run and suitable rinse times should be used to reduce them. The rinse times necessary for a particular element should be estimated prior to analysis. This may be achieved by aspirating a standard containing elements corresponding to 10 times the upper end of the linear range for a normal sample analysis period, followed by analysis of the rinse blank at designated intervals. The length of time required to reduce analyte signals to within a factor of 10 of the method detection limit, should be noted. Memory interferences may also be assessed within an analytical run by using a minimum of three replicate integrations for data acquisition. If the integrated signal values drop consecutively, the analyst should be alerted to the possibility of a memory effect, and should examine the analyte concentration in the previous sample to identify if this was high. If a memory interference is suspected, the sample should be reanalyzed after a long rinse period. In the determination of mercury, which suffers from severe memory effects, the addition of 100 µg/L gold will effectively rinse 5 µg/L mercury in approximately two minutes. Higher concentrations will require a longer rinse time.

5.0 SAFETY

5.1 The toxicity or carcinogenicity of reagents used in this method have not been fully established. Each chemical should be regarded as a potential health hazard and exposure to these compounds should be as low as reasonably achievable. Each laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method.[5,8] A reference file of material data handling sheets should also be available to all personnel involved in the chemical analysis. Specifically, concentrated nitric and hydrochloric acids present various hazards and are moderately toxic and extremely irritating to skin and mucus membranes. Use these reagents in a fume hood whenever possible and if eye or skin contact occurs, flush with large volumes of water. Always wear safety glasses or a shield for eye protection, protective clothing and observe proper mixing when working with these reagents.

5.2 The acidification of samples containing reactive materials may result in the release of toxic gases, such as cyanides or sulfides. Acidification of samples should be done in a fume hood.

5.3 All personnel handling environmental samples known to contain or to have been

in contact with human waste should be immunized against known disease causative agents.

5.4 Analytical plasma sources emit radiofrequency radiation in addition to intense UV radiation. Suitable precautions should be taken to protect personnel from such hazards. The inductively coupled plasma should only be viewed with proper eye protection from UV emissions.

5.5 It is the responsibility of the user of this method to comply with relevant disposal and waste regulations. For guidance see Sections 14.0 and 15.0.

6.0 EQUIPMENT AND SUPPLIES

6.1 Inductively coupled plasma mass spectrometer:

6.1.1 Instrument capable of scanning the mass range 5-250 amu with a minimum resolution capability of 1 amu peak width at 5% peak height. Instrument may be fitted with a conventional or extended dynamic range detection system.

Note: If an electron multiplier detector is being used, precautions should be taken, where necessary, to prevent exposure to high ion flux. Otherwise changes in instrument response or damage to the multiplier may result.

6.1.2 Radio-frequency generator compliant with FCC regulations.

6.1.3 Argon gas supply - High purity grade (99.99%). When analyses are conducted frequently, liquid argon is more economical and requires less frequent replacement of tanks than compressed argon in conventional cylinders (Section 4.1.3).

6.1.4 A variable-speed peristaltic pump is required for solution delivery to the nebulizer.

6.1.5 A mass-flow controller on the nebulizer gas supply is required. A water-cooled spray chamber may be of benefit in reducing some types of interferences (e.g., from polyatomic oxide species).

6.1.6 If an electron multiplier detector is being used, precautions should be taken, where necessary, to prevent exposure to high ion flux. Otherwise changes in instrument response or damage to the multiplier may result. Samples having high concentrations of elements beyond the linear range of the instrument and with isotopes falling within scanning windows should be diluted prior to analysis.

6.2 Analytical balance, with capability to measure to 0.1 mg, for use in weighing solids, for preparing standards, and for determining dissolved solids in digests or extracts.

6.3 A temperature adjustable hot plate capable of maintaining a temperature of 95°C.

6.4 (Optional) A temperature adjustable block digester capable of maintaining a temperature of 95°C and equipped with 250 mL constricted digestion tubes.

6.5 (Optional) A steel cabinet centrifuge with guard bowl, electric timer and brake.

6.6 A gravity convection drying oven with thermostatic control capable of maintaining 105°C ± 5°C.

6.7 (Optional) An air displacement pipetter capable of delivering volumes ranging from 0.1-2500 µL with an assortment of high quality disposable pipet tips.

6.8 Mortar and pestle, ceramic or nonmetallic material.

6.9 Polypropylene sieve, 5-mesh (4 mm opening).

6.10 Labware - For determination of trace levels of elements, contamination and loss are of prime consideration. Potential contamination sources include improperly cleaned laboratory apparatus and general contamination within the laboratory environment from dust, etc. A clean laboratory work area designated for trace element sample handling must be used. Sample containers can introduce positive and negative errors in the determination of trace elements by (1) contributing contaminants through surface desorption or leaching, (2) depleting element concentrations through adsorption processes. All reusable labware (glass, quartz, polyethylene, PTFE, FEP, etc.) should be sufficiently clean for the task objectives. Several procedures found to provide clean labware include soaking overnight and thoroughly washing with laboratory-grade detergent and water, rinsing with tap water, and soaking for four hours or more in 20% (V/V) nitric acid or a mixture of dilute nitric and hydrochloric acid (1+2+9), followed by rinsing with reagent grade water and storing clean.

Note: Chromic acid must not be used for cleaning glassware.

6.10.1 Glassware - Volumetric flasks, graduated cylinders, funnels and centrifuge tubes (glass and/or metal free plastic).

6.10.2 Assorted calibrated pipettes.

6.10.3 Conical Phillips beakers (Corning 1080-250 or equivalent), 250 mL with 50 mm watch glasses.

6.10.4 Griffin beakers, 250 mL with 75 mm watch glasses and (optional) 75 mm ribbed watch glasses.

6.10.5 (Optional) PTFE and/or quartz beakers, 250 mL with PTFE covers.

6.10.6 Evaporating dishes or high-form crucibles, porcelain, 100 mL capacity.

6.10.7 Narrow-mouth storage bottles, FEP (fluorinated ethylene propylene) with ETFE (ethylene tetrafluorethylene) screw closure, 125-250 mL capacities.

6.10.8 One-piece stem FEP wash bottle with screw closure, 125 mL capacity.

7.0 REAGENTS AND STANDARDS

7.1 Reagents may contain elemental impurities that might affect the integrity of analytical data. Owing to the high sensitivity of ICP-MS, high-purity reagents should be used whenever possible. All acids used for this method must be of ultra high-purity grade. Suitable acids are available from a number of manufacturers or may be prepared by sub-boiling distillation. Nitric acid is preferred for ICP-MS in order to minimize polyatomic ion interferences. Several polyatomic ion interferences result when hydrochloric acid is used (Table 2), however, it should be noted that hydrochloric acid is required to maintain stability in solutions containing antimony and silver. When hydrochloric acid is used, corrections for the chloride polyatomic ion interferences must be applied to all data.

7.1.1 Nitric acid, concentrated (sp.gr. 1.41).

7.1.2 Nitric acid (1+1) - Add 500 mL conc. nitric acid to 400 mL of regent grade water and dilute to 1 L.

7.1.3 Nitric acid (1+9) - Add 100 mL conc. nitric acid to 400 mL of reagent grade water and dilute to 1 L.

7.1.4 Hydrochloric acid, concentrated (sp.gr. 1.19).

7.1.5 Hydrochloric acid (1+1) - Add 500 mL conc. hydrochloric acid to 400 mL of reagent grade water and dilute to 1 L.

7.1.6 Hydrochloric acid (1+4) - Add 200 mL conc. hydrochloric acid to 400 mL of reagent grade water and dilute to 1 L.

7.1.7 Ammonium hydroxide, concentrated (sp.gr. 0.902).

7.1.8 Tartaric acid (CASRN 87-69-4).

7.2 Reagent water - All references to reagent grade water in this method refer to ASTM Type I water (ASTM D1193).[9] Suitable water may be prepared by passing distilled water through a mixed bed of anion and cation exchange resins.

7.3 Standard Stock Solutions - Stock standards may be purchased from a reputable commercial source or prepared from ultra high-purity grade chemicals or metals (99.99-99.999% pure). All salts should be dried for one hour at 105°C, unless otherwise specified. Stock solutions should be stored in FEP bottles. Replace stock standards when succeeding dilutions for preparation of the multielement stock standards can not be verified.

CAUTION: Many metal salts are extremely toxic if inhaled or swallowed. Wash hands thoroughly after handling.

The following procedures may be used for preparing standard stock solutions:

Note: Some metals, particularly those which form surface oxides require cleaning prior to being weighed. This may be achieved by pickling the surface of the metal in acid. An amount in excess of the desired weight should be pickled repeatedly, rinsed with water, dried and weighed until the desired weight is achieved.

7.3.1 Aluminum solution, stock 1 mL = 1000 µg Al: Pickle aluminum metal in warm (1+1) HCl to an exact weight of 0.100 g. Dissolve in 10 mL conc. HCl and 2 mL conc. nitric acid, heating to effect solution. Continue heating until volume is reduced to 4 mL. Cool and add 4 mL reagent grade water. Heat until the volume is reduced to 2 mL. Cool and dilute to 100 mL with reagent grade water.

7.3.2 Antimony solution, stock 1 mL = 1000 µg Sb: Dissolve 0.100 g antimony powder in 2 mL (1+1) nitric acid and 0.5 mL conc. hydrochloric acid, heating to effect solution. Cool, add 20 mL reagent grade water and 0.15 g tartaric acid. Warm the solution to dissolve the white precipitate. Cool and dilute to 100 mL with reagent grade water.

7.3.3 Arsenic solution, stock 1 mL = 1000 µg As: Dissolve 0.1320 g As_2O_3 in a mixture of 50 mL reagent grade water and 1 mL conc. ammonium hydroxide. Heat gently to dissolve. Cool and acidify the solution with 2 mL conc. nitric acid. Dilute to 100 mL with reagent grade water.

7.3.4 Barium solution, stock 1 mL = 1000 µg Ba: Dissolve 0.1437 g $BaCO_3$ in a solution mixture of 10 mL reagent grade water and 2 mL conc. nitric acid. Heat and stir to effect solution and degassing. Dilute to 100 mL with reagent grade water.

7.3.5 Beryllium solution, stock 1 mL = 1000 µg Be: Dissolve 1.965 g $BeSO_4 \bullet 4H_2O$ (DO NOT DRY) in 50 mL reagent grade water. Add 1 mL conc. nitric acid. Dilute to 100 mL with reagent grade water.

7.3.6 Bismuth solution, stock 1 mL = 1000 µg Bi: Dissolve 0.1115 g Bi_2O_3 in 5 mL conc. nitric acid. Heat to effect solution. Cool and dilute to 100 mL with reagent grade water.

7.3.7 Cadmium solution, stock 1 mL = 1000 µg Cd: Pickle cadmium metal in (1+9) nitric acid to an exact weight of 0.100 g. Dissolve in 5 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with reagent grade water.

7.3.8 Chromium solution, stock 1 mL = 1000 µg Cr: Dissolve 0.1923 g CrO_3 in a solution mixture of 10 mL reagent grade water and 1 mL conc. nitric

acid. Dilute to 100 mL with reagent grade water.

7.3.9 Cobalt solution, stock 1 mL = 1000 µg Co: Pickle cobalt metal in (1+9) nitric acid to an exact weight of 0.100 g. Dissolve in 5 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with reagent grade water.

7.3.10 Copper solution, stock 1 mL = 1000 µg Cu: Pickle copper metal in (1+9) nitric acid to an exact weight of 0.100 g. Dissolve in 5 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with reagent grade water.

7.3.11 Gold solution, stock 1 mL = 1000 µg Au: Dissolve 0.100 g high purity (99.9999%) Au shot in 10 mL of hot conc. nitric acid by dropwise addition of 5 mL conc. HCl and then reflux to expel oxides of nitrogen and chlorine. Cool and dilute to 100 mL with reagent grade water.

7.3.12 Indium solution, stock 1 mL = 1000 µg In: Pickle indium metal in (1+1) nitric acid to an exact weight of 0.100 g. Dissolve in 10 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with reagent grade water.

7.3.13 Lead solution, stock 1 mL = 1000 µg Pb: Dissolve 0.1599 g $PbNO_3$ in 5 mL (1+1) nitric acid. Dilute to 100 mL with reagent grade water.

7.3.14 Magnesium solution, stock 1 mL = 1000 µg Mg: Dissolve 0.1658 g MgO in 10 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with reagent grade water.

7.3.15 Manganese solution, stock 1 mL = 1000 µg Mn: Pickle manganese flake in (1+9) nitric acid to an exact weight of 0.100 g. Dissolve in 5 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with reagent grade water.

7.3.16 Mercury solution, stock, 1 mL = 1000 µg Hg: DO NOT DRY. **CAUTION**: highly toxic element. Dissolve 0.1354 g $HgCl_2$ in reagent water. Add 5.0 mL concentrated HNO_3 and dilute to 100 mL with reagent water.

7.3.17 Molybdenum solution, stock 1 mL = 1000 µg Mo: Dissolve 0.1500 g MoO_3 in a solution mixture of 10 mL reagent grade water and 1 mL conc. ammonium hydroxide., heating to effect solution. Cool and dilute to 100 mL with reagent grade water.

7.3.18 Nickel solution, stock 1 mL = 1000 µg Ni: Dissolve 0.100 g nickel powder in 5 mL conc. nitric acid, heating to effect solution. Cool and dilute to 100 mL with reagent grade water.

7.3.19 Scandium solution, stock 1 mL = 1000 µg Sc: Dissolve 0.1534 g Sc_2O_3 in 5 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to

100 mL with reagent grade water.

7.3.20 Selenium solution, stock 1 mL = 1000 µg Se: Dissolve 0.1405 g SeO_2 in 20 mL ASTM Type I water. Dilute to 100 mL with reagent grade water.

7.3.21 Silver solution, stock 1 mL = 1000 µg Ag: Dissolve 0.100 g silver metal in 5 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with reagent grade water. Store in dark container.

7.3.22 Terbium solution, stock 1 mL = 1000 µg Tb: Dissolve 0.1176 g Tb_4O_7 in 5 mL conc. nitric acid, heating to effect solution. Cool and dilute to 100 mL with reagent grade water.

7.3.23 Thallium solution, stock 1 mL = 1000 µg Tl: Dissolve 0.1303 g $TlNO_3$ in a solution mixture of 10 mL reagent grade water and 1 mL conc. nitric acid. Dilute to 100 mL with reagent grade water.

7.3.24 Thorium solution, stock 1 mL = 1000 µg Th: Dissolve 0.2380 g $Th(NO_3)_4 \cdot 4H_2O$ (DO NOT DRY) in 20 mL reagent grade water. Dilute to 100 mL with reagent grade water.

7.3.25 Uranium solution, stock 1 mL = 1000 µg U: Dissolve 0.2110 g $UO_2(NO_3)_2 \cdot 6H_2O$ (DO NOT DRY) in 20 mL reagent grade water and dilute to 100 mL with reagent grade water.

7.3.26 Vanadium solution, stock 1 mL = 1000 µg V: Pickle vanadium metal in (1+9) nitric acid to an exact weight of 0.100 g. Dissolve in 5 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with reagent grade water.

7.3.27 Yttrium solution, stock 1 mL = 1000 µg Y: Dissolve 0.1270 g Y_2O_3 in 5 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with reagent grade water.

7.3.28 Zinc solution, stock 1 mL = 1000 µg Zn: Pickle zinc metal in (1+9) nitric acid to an exact weight of 0.100 g. Dissolve in 5 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with reagent grade water.

7.4 Multielement Stock Standard Solutions - Care must be taken in the preparation of multielement stock standards that the elements are compatible and stable. Originating element stocks should be checked for the presence of impurities which might influence the accuracy of the standard. Freshly prepared standards should be transferred to acid cleaned, not previously used FEP fluorocarbon bottles for storage and monitored periodically for stability. The following combinations of elements are suggested:

Standard Solution A		Standard Solution B
Aluminum	Mercury	Barium
Antimony	Molybdenum	Silver
Arsenic	Nickel	
Beryllium	Selenium	
Cadmium	Thallium	
Chromium	Thorium	
Cobalt	Uranium	
Copper	Vanadium	
Lead	Zinc	
Manganese		

Except for selenium and mercury, multielement stock standard solutions A and B (1 mL = 10 µg) may be prepared by diluting 1.0 mL of each single element stock standard in the combination list to 100 mL with reagent water containing 1% (v/v) nitric acid. For mercury and selenium in solution A, aliquots of 0.05 mL and 5.0 mL of the respective stock standards should be diluted to the specified 100 mL (1 ml = 0.5 µg Hg and 50 µg Se). Replace the multielement stock standards when succeeding dilutions for preparation of the calibration standards cannot be verified with the quality control sample.

7.4.1 Preparation of calibration standards - fresh multielement calibration standards should be prepared every two weeks or as needed. Dilute each of the stock multielement standard solutions A and B to levels appropriate to the operating range of the instrument using reagent water containing 1% (v/v) nitric acid. The element concentrations in the standards should be sufficiently high to produce good measurement precision and to accurately define the slope of the response curve. Depending on the sensitivity of the instrument, concentrations ranging from 10-200 µg/L are suggested, except mercury, which should be limited to ≤5 µg/L. It should be noted the selenium concentration is always a factor of 5 greater than the other analytes. If the direct addition procedure is being used (Method A, Section 10.3), add internal standards (Section 7.5) to the calibration standards and store in FEP bottles. Calibration standards should be verified initially using a quality control sample (Section 7.8).

7.5 Internal Standards Stock Solution - 1 mL = 100 µg. Dilute 10 mL of scandium, yttrium, indium, terbium and bismuth stock standards (Section 7.3) to 100 mL with reagent water, and store in a FEP bottle. Use this solution concentrate for addition to blanks, calibration standards and samples, or dilute by an appropriate amount using 1% (v/v) nitric acid, if the internal standards are being added by peristaltic pump (Method B, Section 10.3).

Note: If mercury is to be determined by the "direct analysis" procedure, add an aliquot of the gold stock standard (Section 7.3.11) to the internal standard solution sufficient to provide a concentration of 100 µg/L in final the dilution of all blanks, calibration standards, and samples.

7.6 Blanks - Three types of blanks are required for this method. A calibration blank is used to establish the analytical calibration curve, the laboratory reagent blank is used to assess possible contamination from the sample preparation procedure and to assess spectral background and the rinse blank is used to flush the instrument between samples in order to reduce memory interferences.

7.6.1 Calibration blank - Consists of 1% (v/v) nitric acid in reagent grade water. If the direct addition procedure (Method A, Section 10.3) is being used, add internal standards.

7.6.2 Laboratory reagent blank (LRB) - Must contain all the reagents in the same volumes as used in processing the samples. The LRB must be carried through the same entire preparation scheme as the samples including digestion, when applicable. If the direct addition procedure (Method A, Section 10.3) is being used, add internal standards to the solution after preparation is complete.

7.6.3 Rinse blank - Consists of 2% (v/v) nitric acid in reagent grade water.

Note: If mercury is to be determined by the "direct analysis" procedure, add gold (Section 7.3.11) to the rinse blank to a concentration of 100 µg/L.

7.7 Tuning Solution - This solution is used for instrument tuning and mass calibration prior to analysis. The solution is prepared by mixing beryllium, magnesium, cobalt, indium and lead stock solutions (Section 7.3) in 1% (v/v) nitric acid to produce a concentration of 100 µg/L of each element. Internal standards are not added to this solution. (Depending on the sensitivity of the instrument, this solution may need to be diluted 10-fold.)

7.8 Quality Control Sample (QCS) - The QCS should be obtained from a source outside the laboratory. The concentration of the QCS solution analyzed will depend on the sensitivity of the instrument. To prepare the QCS dilute an appropriate aliquot of analytes to a concentration ≤100 µg/L in 1% (v/v) nitric acid. Because of lower sensitivity, selenium may be diluted to a concentration of <500 µg/L, however, in all cases, mercury should be limited to a concentration of ≤5 µg/L. If the direct addition procedure (Method A, Section 10.3) is being used, add internal standards after dilution, mix and store in a FEP bottle. The QCS should be analyzed as needed to meet data-quality needs and a fresh solution should be prepared quarterly or more frequently as needed.

7.9 Laboratory Fortified Blank (LFB) - To an aliquot of LRB, add aliquots from multielement stock standards A and B (Section 7.4) to prepared the LFB. Depending on the sensitivity of the instrument, the fortified concentration used should range from 40-100 µg/L for each analyte, except selenium and mercury. For selenium the concentration should range from 200-500 µg/L, while the concentration range mercury should be limited to 2-5 µg/L. The LFB must be carried through the same entire preparation scheme as the samples including sample digestion, when applicable. If the direct addition procedure (Method A, Section 10.3) is being used, add internal standards to this solution after

preparation has been completed.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 Prior to the collection of an aqueous sample, consideration should be given to the type of data required, (i.e., dissolved or total recoverable), so that appropriate preservation and pretreatment steps can be taken. The pH of all aqueous samples **must** be tested immediately prior to aliquoting for processing or "direct analysis" to ensure the sample has been properly preserved. If properly acid preserved, the sample can be held up to 6 months before analysis.

8.2 For the determination of dissolved elements, the sample must be filtered through a 0.45 µm pore diameter membrane filter at the time of collection or as soon thereafter as practically possible. Use a portion of the sample to rinse the filter flask, discard this portion and collect the required volume of filtrate. Acidify the filtrate with (1+1) nitric acid immediately following filtration to pH <2.

8.3 For the determination of total recoverable elements in aqueous samples, samples are **not** filtered, but acidified with (1+1) nitric acid to pH <2 (normally, 3 mL of (1+1) acid per liter of sample is sufficient for most ambient and drinking water samples). Preservation may be done at the time of collection, however, to avoid the hazards of strong acids in the field, transport restrictions, and possible contamination it is recommended that the samples be returned to the laboratory within two weeks of collection and acid preserved upon receipt in the laboratory. Following acidification, the sample should be mixed, held for 16 hours, and then verified to be pH <2 just prior withdrawing an aliquot for processing or "direct analysis". If for some reason such as high alkalinity the sample pH is verified to be >2, more acid must be added and the sample held for 16 hours until verified to be pH <2. See Section 8.1.

Note: When the nature of the sample is either unknown or known to be hazardous, acidification should be done in a fume hood. See Section 5.2.

8.4 Solid samples require no preservation prior to analysis other than storage at 4°C. There is no established holding time limitation for solid samples.

8.5 For aqueous samples, a field blank should be prepared and analyzed as required by the data user. Use the same container and acid as used in sample collection.

9.0 QUALITY CONTROL

9.1 Each laboratory using this method is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability, and the periodic analysis of laboratory reagent blanks, fortified blanks and calibration solutions as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of the data thus generated.

9.2 Initial Demonstration of Performance (mandatory)

9.2.1 The initial demonstration of performance is used to characterize instrument performance (determination of linear calibration ranges and analysis of quality control samples) and laboratory performance (determination of method detection limits) prior to analyses conducted by this method.

9.2.2 Linear calibration ranges - Linear calibration ranges are primarily detector limited. The upper limit of the linear calibration range should be established for each analyte by determining the signal responses from a minimum of three different concentration standards, one of which is close to the upper limit of the linear range. Care should be taken to avoid potential damage to the detector during this process. The linear calibration range which may be used for the analysis of samples should be judged by the analyst from the resulting data. The upper LDR limit should be an observed signal no more than 10% below the level extrapolated from lower standards. Determined sample analyte concentrations that are greater than 90% of the determined upper LDR limit must be diluted and reanalyzed. The LDRs should be verified whenever, in the judgement of the analyst, a change in analytical performance caused by either a change in instrument hardware or operating conditions would dictate they be redetermined.

9.2.3 Quality control sample (QCS) - When beginning the use of this method, on a quarterly basis or as required to meet data-quality needs, verify the calibration standards and acceptable instrument performance with the preparation and analyses of a QCS (Section 7.8). To verify the calibration standards the determined mean concentration from three analyses of the QCS must be within ±10% of the stated QCS value. If the QCS is used for determining acceptable on-going instrument performance, analysis of the QCS prepared to a concentration of 100 µg/L must be within ±10% of the stated value or within the acceptance limits listed in Table 8, whichever is the greater. (If the QCS is not within the required limits, an immediate second analysis of the QCS is recommended to confirm unacceptable performance.) If the calibration standards and/or acceptable instrument performance cannot be verified, the source of the problem must be identified and corrected before either proceeding on with the initial determination of method detection limits or continuing with on-going analyses.

9.2.4 Method detection limits (MDL) should be established for all analytes, using reagent water (blank) fortified at a concentration of two to five times the estimated detection limit.[7] To determine MDL values, take seven replicate aliquots of the fortified reagent water and process through the entire analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$MDL = (t) \times (S)$$

where:

t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates]

S = standard deviation of the replicate analyses

Note: If additional confirmation is desired, reanalyze the seven replicate aliquots on two more nonconsecutive days and again calculate the MDL values for each day. An average of the three MDL values for each analyte may provide for a more appropriate MDL estimate. If the relative standard deviation (RSD) from the analyses of the seven aliquots is <10%, the concentration used to determine the analyte MDL may have been inappropriately high for the determination. If so, this could result in the calculation of an unrealistically low MDL. Concurrently, determination of MDL in reagent water represents a best case situation and does not reflect possible matrix effects of real world samples. However, successful analyses of LFMs (Section 9.4) can give confidence to the MDL value determined in reagent water. Typical single laboratory MDL values using this method are given in Table 7.

The MDLs must be sufficient to detect analytes at the required levels according to compliance monitoring regulation (Section 1.2). MDLs should be determined annually, when a new operator begins work or whenever, in the judgement of the analyst, a change in analytical performance caused by either a change in instrument hardware or operating conditions would dictate they be redetermined.

9.3 Assessing Laboratory Performance (mandatory)

9.3.1 Laboratory reagent blank (LRB) - The laboratory must analyze at least one LRB (Section 7.6.2) with each batch of 20 or fewer of samples of the same matrix. LRB data are used to assess contamination from the laboratory environment and to characterize spectral background from the reagents used in sample processing. LRB values that exceed the MDL indicate laboratory or reagent contamination should be suspected. When LRB values constitute 10% or more of the analyte level determined for a sample or is 2.2 times the analyte MDL whichever is greater, fresh aliquots of the samples must be prepared and analyzed again for the affected analytes after the source of contamination has been corrected and acceptable LRB values have been obtained.

9.3.2 Laboratory fortified blank (LFB) - The laboratory must analyze at least one LFB (Section 7.9) with each batch of samples. Calculate accuracy as percent recovery using the following equation:

$$R = \frac{LFB - LRB}{s} \times 100$$

where:

R = percent recovery
LFB = laboratory fortified blank
LRB = laboratory reagent blank
s = concentration equivalent of analyte added to fortify the LBR solution

If the recovery of any analyte falls outside the required control limits of 85-115%, that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

9.3.3 The laboratory must use LFB analyses data to assess laboratory performance against the required control limits of 85-115% (Section 9.3.2). When sufficient internal performance data become available (usually a minimum of 20-30 analyses), optional control limits can be developed from the mean percent recovery (x) and the standard deviation (S) of the mean percent recovery. These data can be used to establish the upper and lower control limits as follows:

$$\text{UPPER CONTROL LIMIT} = x + 3S$$
$$\text{LOWER CONTROL LIMIT} = x - 3S$$

The optional control limits must be equal to or better than the required control limits of 85-115%. After each five to ten new recovery measurements, new control limits can be calculated using only the most recent 20-30 data points. Also, the standard deviation (S) data should be used to establish an on-going precision statement for the level of concentrations included in the LFB. These data must be kept on file and be available for review.

9.3.4 Instrument performance - For all determinations the laboratory must check instrument performance and verify that the instrument is properly calibrated on a continuing basis. To verify calibration run the calibration blank and calibration standards as surrogate samples immediately following each calibration routine, after every ten analyses and at the end of the sample run. The results of the analyses of the standards will indicate whether the calibration remains valid. The analysis of all analytes within the standard solutions must be within ±10% of calibration. If the calibration cannot be verified within the specified limits, the instrument must be recalibrated. (The instrument responses from the calibration check may be used for recalibration purposes, however, it must be verified before continuing sample analysis.) If the continuing calibration check is not confirmed within ±15%, the previous 10 samples must be reanalyzed after recalibration. If the sample matrix is responsible for the calibration drift, it is recommended that the previous 10 samples are reanalyzed in groups of five between calibration checks to prevent a similar drift situation from occurring.

9.4 Assessing Analyte Recovery and Data Quality

9.4.1 Sample homogeneity and the chemical nature of the sample matrix can affect analyte recovery and the quality of the data. Taking separate aliquots from the sample for replicate and fortified analyses can in some cases assess the effect. Unless otherwise specified by the data user, laboratory or program, the following laboratory fortified matrix (LFM) procedure (Section 9.4.2) is required.

9.4.2 The laboratory must add a known amount of analyte to a minimum of 10% of the routine samples. In each case the LFM aliquot must be a duplicate of the aliquot used for sample analysis and for total recoverable determinations added prior to sample preparation. For water samples, the added analyte concentration must be the same as that used in the laboratory fortified blank (Section 7.9). For solid samples, the concentration added should be 100 mg/kg equivalent (200 µg/L in the analysis solution) except silver which should be limited to 50 mg/kg (Section 1.8). Over time, samples from all routine sample sources should be fortified.

9.4.3 Calculate the percent recovery for each analyte, corrected for background concentrations measured in the unfortified sample, and compare these values to the designated LFM recovery range of 70-130%. Recovery calculations are not required if the concentration of the analyte added is less than 30% of the sample background concentration. Percent recovery may be calculated in units appropriate to the matrix, using the following equation:

$$R = \frac{C_s - C}{s} \times 100$$

where:

R = percent recovery
C_s = fortified sample concentration
C = sample background concentration
s = concentration equivalent of analyte added to fortify the sample

9.4.4 If recovery of any analyte falls outside the designated range and laboratory performance for that analyte is shown to be in control (Section 9.3), the recovery problem encountered with the fortified sample is judged to be matrix related, not system related. The data user should be informed that the result for that analyte in the unfortified sample is suspect due to either the heterogeneous nature of the sample or an uncorrected matrix effect.

9.4.5 Internal standards responses - The analyst is expected to monitor the responses from the internal standards throughout the sample set being

analyzed. Ratios of the internal standards responses against each other should also be monitored routinely. This information may be used to detect potential problems caused by mass dependent drift, errors incurred in adding the internal standards or increases in the concentrations of individual internal standards caused by background contributions from the sample. The absolute response of any one internal standard must not deviate more than 60-125% of the original response in the calibration blank. If deviations greater than these are observed, flush the instrument with the rinse blank and monitor the responses in the calibration blank. If the responses of the internal standards are now within the limit, take a fresh aliquot of the sample, dilute by a further factor of two, add the internal standards and reanalyze. If after flushing the response of the internal standards in the calibration blank are out of limits, terminate the analysis and determine the cause of the drift. Possible causes of drift may be a partially blocked sampling cone or a change in the tuning condition of the instrument.

10.0 CALIBRATION AND STANDARDIZATION

10.1 Operating conditions - Because of the diversity of instrument hardware, no detailed instrument operating conditions are provided. The analyst is advised to follow the recommended operating conditions provided by the manufacturer. It is the responsibility of the analyst to verify that the instrument configuration and operating conditions satisfy the analytical requirements and to maintain quality control data verifying instrument performance and analytical results. Instrument operating conditions which were used to generate precision and recovery data for this method (Section 13.0) are included in Table 6.

10.2 Precalibration routine - The following precalibration routine must be completed prior to calibrating the instrument until such time it can be documented with periodic performance data that the instrument meets the criteria listed below without daily tuning.

10.2.1 Initiate proper operating configuration of instrument and data system. Allow a period of not less than 30 minutes for the instrument to warm up. During this process conduct mass calibration and resolution checks using the tuning solution. Resolution at low mass is indicated by magnesium isotopes 24, 25, and 26. Resolution at high mass is indicated by lead isotopes 206, 207, and 208. For good performance adjust spectrometer resolution to produce a peak width of approximately 0.75 amu at 5% peak height. Adjust mass calibration if it has shifted by more than 0.1 amu from unit mass.

10.2.2 Instrument stability must be demonstrated by running the tuning solution (Section 7.7) a minimum of five times with resulting relative standard deviations of absolute signals for all analytes of less than 5%.

10.3 Internal Standardization - Internal standardization must be used in all analyses to correct for instrument drift and physical interferences. A list of acceptable

internal standards is provided in Table 3. For full mass range scans, a minimum of three internal standards must be used. Procedures described in this method for general application, detail the use of five internal standards; scandium, yttrium, indium, terbium and bismuth. These were used to generate the precision and recovery data attached to this method. Internal standards must be present in all samples, standards and blanks at identical levels. This may be achieved by directly adding an aliquot of the internal standards to the CAL standard, blank or sample solution (Method A, Section 10.3), or alternatively by mixing with the solution prior to nebulization using a second channel of the peristaltic pump and a mixing coil (Method B, Section 10.3). The concentration of the internal standard should be sufficiently high that good precision is obtained in the measurement of the isotope used for data correction and to minimize the possibility of correction errors if the internal standard is naturally present in the sample. Depending on the sensitivity of the instrument, a concentration range of 20-200 µg/L of each internal standard is recommended. Internal standards should be added to blanks, samples and standards in a like manner, so that dilution effects resulting from the addition may be disregarded.

10.4 Calibration - Prior to initial calibration, set up proper instrument software routines for quantitative analysis. The instrument must be calibrated using one of the internal standard routines (Method A or B) described in Section 10.3. The instrument must be calibrated for the analytes to be determined using the calibration blank (Section 7.6.1) and calibration standards A and B (Section 7.4.1) prepared at one or more concentration levels. A minimum of three replicate integrations are required for data acquisition. Use the average of the integrations for instrument calibration and data reporting.

10.5 The rinse blank should be used to flush the system between solution changes for blanks, standards and samples. Allow sufficient rinse time to remove traces of the previous sample (Section 4.1.5). Solutions should be aspirated for 30 seconds prior to the acquisition of data to allow equilibrium to be established.

11.0 PROCEDURE

11.1 Aqueous Sample Preparation - Dissolved Analytes

11.1.1 For the determination of dissolved analytes in ground and surface waters, pipet an aliquot (≥20 mL) of the filtered, acid preserved sample into a 50 mL polypropylene centrifuge tube. Add an appropriate volume of (1+1) nitric acid to adjust the acid concentration of the aliquot to approximate a 1% (v/v) nitric acid solution (e.g., add 0.4 mL (1+1) HNO_3 to a 20 mL aliquot of sample). If the direct addition procedure (Method A, Section 10.3) is being used, add internal standards, cap the tube and mix. The sample is now ready for analysis (Section 1.2). Allowance for sample dilution should be made in the calculations.

Note: If a precipitate is formed during acidification, transport, or storage, the sample aliquot must be treated using the procedure in Section 11.2 prior to analysis.

11.2 Aqueous Sample Preparation - Total Recoverable Analytes

11.2.1 For the "direct analysis" of total recoverable analytes in drinking water samples containing turbidity <1 NTU, treat an unfiltered acid preserved sample aliquot using the sample preparation procedure described in Section 11.1.1 while making allowance for sample dilution in the data calculation. For the determination of total recoverable analytes in all other aqueous samples or for preconcentrating drinking water samples prior to analysis follow the procedure given in Sections 11.2.2 through 11.2.8.

11.2.2 For the determination of total recoverable analytes in aqueous samples (other than drinking water with <1 NTU turbidity), transfer a 100 mL (±1 mL) aliquot from a well mixed, acid preserved sample to a 250 mL Griffin beaker (Sections 1.2, 1.3, 1.7, and 1.8). (When necessary, smaller sample aliquot volumes may be used.)

Note: If the sample contains undissolved solids >1%, a well mixed, acid preserved aliquot containing no more than 1 g particulate material should be cautiously evaporated to near 10 mL and extracted using the acid-mixture procedure described in Sections 11.3.3 through 11.3.7.

11.2.3 Add 2 mL (1+1) nitric acid and 1.0 mL of (1+1) hydrochloric acid to the beaker containing the measured volume of sample. Place the beaker on the hot plate for solution evaporation. The hot plate should be located in a fume hood and previously adjusted to provide evaporation at a temperature of approximately but no higher than 85°C. (See the following note.) The beaker should be covered with an elevated watch glass or other necessary steps should be taken to prevent sample contamination from the fume hood environment.

Note: For proper heating adjust the temperature control of the hot plate such that an uncovered Griffin beaker containing 50 mL of water placed in the center of the hot plate can be maintained at a temperature approximately but no higher than 85°C. (Once the beaker is covered with a watch glass the temperature of the water will rise to approximately 95°C.)

11.2.4 Reduce the volume of the sample aliquot to about 20 mL by gentle heating at 85°C. DO NOT BOIL. This step takes about two hours for a 100 mL aliquot with the rate of evaporation rapidly increasing as the sample volume approaches 20 mL. (A spare beaker containing 20 mL of water can be used as a gauge.)

11.2.5 Cover the lip of the beaker with a watch glass to reduce additional evaporation and gently reflux the sample for 30 minutes. (Slight boiling may occur, but vigorous boiling must be avoided to prevent loss of the HCl-H_2O azeotrope.)

11.2.6 Allow the beaker to cool. Quantitatively transfer the sample solution to

a 50 mL volumetric flask or 50 mL class A stoppered graduated cylinder, make to volume with reagent water, stopper and mix.

11.2.7 Allow any undissolved material to settle overnight, or centrifuge a portion of the prepared sample until clear. (If after centrifuging or standing overnight the sample contains suspended solids that would clog the nebulizer, a portion of the sample may be filtered for their removal prior to analysis. However, care should be exercised to avoid potential contamination from filtration.)

11.2.8 Prior to analysis, adjust the chloride concentration by pipetting 20 mL of the prepared solution into a 50 mL volumetric flask, dilute to volume with reagent water and mix. (If the dissolved solids in this solution are >0.2%, additional dilution may be required to prevent clogging of the extraction and/or skimmer cones. If the direct addition procedure (Method A, Section 10.3) is being used, add internal standards and mix. The sample is now ready for analysis. Because the effects of various matrices on the stability of diluted samples cannot be characterized, all analyses should be performed as soon as possible after the completed preparation.

11.3 Solid Sample Preparation - Total Recoverable Analytes

11.3.1 For the determination of total recoverable analytes in solid samples, mix the sample thoroughly and transfer a portion (>20 g) to tared weighing dish, weigh the sample and record the wet weight (WW). (For samples with <35% moisture a 20 g portion is sufficient. For samples with moisture >35% a larger aliquot 50-100 g is required.) Dry the sample to a constant weight at 60°C and record the dry weight (DW) for calculation of percent solids (Section 12.6). (The sample is dried at 60°C to prevent the loss of mercury and other possible volatile metallic compounds, to facilitate sieving, and to ready the sample for grinding.)

11.3.2 To achieve homogeneity, sieve the dried sample using a 5-mesh polypropylene sieve and grind in a mortar and pestle. (The sieve, mortar and pestle should be cleaned between samples.) From the dried, ground material weigh accurately a representative 1.0 ± 0.01 g aliquot (W) of the sample and transfer to a 250 mL Phillips beaker for acid extraction.

11.3.3 To the beaker add 4 mL of (1+1) HNO_3 and 10 mL of (1+4) HCl. Cover the lip of the beaker with a watch glass. Place the beaker on a hot plate for reflux extraction of the analytes. The hot plate should be located in a fume hood and previously adjusted to provide a reflux temperature of approximately 95°C. (See the following note.)

Note: For proper heating adjust the temperature control of the hot plate such that an uncovered Griffin beaker containing 50 mL of water placed in the center of the hot plate can be maintained at a temperature approximately but no higher than 85°C. (Once the beaker is covered with a watch glass the temperature of the water will rise to approximately

95°C.) Also, a block digester capable of maintaining a temperature of 95°C and equipped with 250 mL constricted volumetric digestion tubes may be substituted for the hot plate and conical beakers in the extraction step.

11.3.4 Heat the sample and gently reflux for 30 minutes. Very slight boiling may occur, however vigorous boiling must be avoided to prevent loss of the HCl-H_2O azeotrope. Some solution evaporation will occur (3-4 mL).

11.3.5 Allow the sample to cool and quantitatively transfer the extract to a 100 mL volumetric flask. Dilute to volume with reagent water, stopper and mix.

11.3.6 Allow the sample extract solution to stand overnight to separate insoluble material or centrifuge a portion of the sample solution until clear. (If after centrifuging or standing overnight the extract solution contains suspended solids that would clog the nebulizer, a portion of the extract solution may be filtered for their removal prior to analysis. However, care should be exercised to avoid potential contamination from filtration.)

11.3.7 Prior to analysis, adjust the chloride concentration by pipetting 20 mL of the prepared solution into a 100 mL volumetric flask, dilute to volume with reagent water and mix. (If the dissolved solids in this solution are >0.2%, additional dilution may be required to prevent clogging of the extraction and/or skimmer cones. If the direct addition procedure (Method A, Section 10.3) is being used, add internal standards and mix. The sample extract is now ready for analysis. Because the effects of various matrices on the stability of diluted samples cannot be characterized, all analyses should be performed as soon as possible after the completed preparation.

Note: Determine the percent solids in the sample for use in calculations and for reporting data on a dry weight basis.

11.4 Sample Analysis

11.4.1 For every new or unusual matrix, it is highly recommended that a semi-quantitative analysis be carried out to screen the sample for elements at high concentration. Information gained from this may be used to prevent potential damage to the detector during sample analysis and to identify elements which may be higher than the linear range. Matrix screening may be carried out by using intelligent software, if available, or by diluting the sample by a factor of 500 and analyzing in a semi-quantitative mode. The sample should also be screened for background levels of all elements chosen for use as internal standards in order to prevent bias in the calculation of the analytical data.

11.4.2 Initiate instrument operating configuration. Tune and calibrate the instrument for the analytes of interest (Section 10.0).

11.4.3 Establish instrument software run procedures for quantitative analysis. For all sample analyses, a minimum of three replicate integrations are required for data acquisition. Use the average of the integrations for data reporting.

11.4.4 All masses which might affect data quality must be monitored during the analytical run. As a minimum, those masses prescribed in Table 4 must be monitored in the same scan as is used for the collection of the data. This information should be used to correct the data for identified interferences.

11.4.5 During the analysis of samples, the laboratory must comply with the required quality control described in Sections 9.3 and 9.4. Only for the determination of dissolved analytes or the "direct analysis" of drinking water with turbidity of <1 NTU is the sample digestion step of the LRB, LFB, and LFM not required.

11.4.6 The rinse blank should be used to flush the system between samples. Allow sufficient time to remove traces of the previous sample or a minimum of one minute (Section 4.1.5). Samples should be aspirated for 30 seconds prior to the collection of data.

11.4.7 Samples having concentrations higher than the established linear dynamic range should be diluted into range and reanalyzed. The sample should first be analyzed for the trace elements in the sample, protecting the detector from the high concentration elements, if necessary, by the selection of appropriate scanning windows. The sample should then be diluted for the determination of the remaining elements. Alternatively, the dynamic range may be adjusted by selecting an alternative isotope of lower natural abundance, provided quality control data for that isotope have been established. The dynamic range must not be adjusted by altering instrument conditions to an uncharacterized state.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 Elemental equations recommended for sample data calculations are listed in Table 5. Sample data should be reported in units of µg/L for aqueous samples or mg/kg dry weight for solid samples. Do not report element concentrations below the determined MDL.

12.2 For data values less than 10, two significant figures should be used for reporting element concentrations. For data values greater than or equal to 10, three significant figures should be used.

12.3 For aqueous samples prepared by total recoverable procedure (Section 11.2), multiply solution concentrations by the dilution factor 1.25. If additional dilutions were made to any samples or an aqueous sample was prepared using the acid-mixture procedure described in Section 11.3, the appropriate factor should be applied to the calculated sample concentrations.

12.4 For total recoverable analytes in solid samples (Section 11.3), round the solution analyte concentrations (µg/L in the analysis solution) as instructed in Section 12.2. Multiply the µ/L concentrations in the analysis solution by the factor 0.005 to calculate the mg/L analyte concentration in the 100 mL extract solution. (If additional dilutions were made to any samples, the appropriate factor should be applied to calculate analyte concentrations in the extract solution.) Report the data up to three significant figures as mg/kg dry-weight basis unless specified otherwise by the program or data user. Calculate the concentration using the equation below:

$$\text{Sample Conc. (mg/kg) dry-weight basis} = \frac{C \times V}{W}$$

where:

C = Concentration in the extract (mg/L)
V = Volume of extract (L, 100 mL = 0.1L)
W = Weight of sample aliquot extracted (g x 0.001 = kg)

Do not report analyte data below the estimated solids MDL or an adjusted MDL because of additional dilutions required to complete the analysis.

12.5 To report percent solids in solid samples (Sect. 11.3) calculate as follows:

$$\% \text{ solids (S)} = \frac{DW}{WW} \times 100$$

where:

DW = Sample weight (g) dried at 60°C
WW = Sample weight (g) before drying

Note: If the data user, program or laboratory requires that the reported percent solids be determined by drying at 105°C, repeat the procedure given in Section 11.3 using a separate portion (>20 g) of the sample and dry to constant weight at 103-105°C.

12.6 Data values should be corrected for instrument drift or sample matrix induced interferences by the application of internal standardization. Corrections for characterized spectral interferences should be applied to the data. Chloride interference corrections should be made on all samples, regardless of the addition of hydrochloric acid, as the chloride ion is a common constituent of environmental samples.

12.7 If an element has more than one monitored isotope, examination of the concentration calculated for each isotope, or the isotope ratios, will provide useful information for the analyst in detecting a possible spectral interference.

Consideration should therefore be given to both primary and secondary isotopes in the evaluation of the element concentration. In some cases, secondary isotopes may be less sensitive or more prone to interferences than the primary recommended isotopes, therefore differences between the results do not necessarily indicate a problem with data calculated for the primary isotopes.

12.8 The QC data obtained during the analyses provide an indication of the quality of the sample data and should be provided with the sample results.

13.0 METHOD PERFORMANCE

13.1 Instrument operating conditions used for single laboratory testing of the method are summarized in Table 6. Total recoverable digestion and "direct analysis" MDLs determined using the procedure described in Section 9.2.4, are listed in Table 7.

13.2 Data obtained from single laboratory testing of the method are summarized in Table 9 for five water samples representing drinking water, surface water, ground water and waste effluent. Samples were prepared using the procedure described in Section 11.2. For each matrix, five replicates were analyzed and the average of the replicates used for determining the sample background concentration for each element. Two further pairs of duplicates were fortified at different concentration levels. For each method element, the sample background concentration, mean percent recovery, the standard deviation of the percent recovery and the relative percent difference between the duplicate fortified samples are listed in Table 8.

13.3 Data obtained from single laboratory testing of the method are summarized in Table 10 for three solid samples consisting of SRM 1645 River Sediment, EPA Hazardous Soil and EPA Electroplating Sludge. Samples were prepared using the procedure described in Section 11.3. For each method element, the sample background concentration, mean percent recovery, the standard deviation of the percent recovery and the relative percent difference between the duplicate fortified samples were determined as for Section 13.2.

13.4 Data obtained from single laboratory testing of the method for drinking water analysis using the "direct analysis" procedure (Section 11.2.1) are given in Table 11. Three drinking water samples of varying hardness collected from Regions 4, 6, and 10 were fortified to contain 1 µg/L of all metal primary contaminants, except selenium, which was added to a concentration of 20 µg/L. For each matrix, four replicate aliquots were analyzed to determine the sample background concentration of each analyte and four fortified aliquots were analyzed to determine mean percent recovery in each matrix. Listed in the Table 11 are the average mean percent recovery of each analyte in the three matrices and the standard deviation of the mean percent recoveries.

13.5 Listed in Table 12 are the regression equations for precision and bias developed from the joint USEPA/Association of Official Analytical Chemists (AOAC) multilaboratory validation study conducted on this method. These equations

were developed from data received from 13 laboratories on reagent water, drinking water and ground water. Listed in Tables 13 and 14, respectively, are the precision and recovery data from a wastewater digestate supplied to all laboratories and from a wastewater of the participant's choice. For a complete review of the study see Reference 11, Section 16.0 of this method.

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions, consult "Less is Better: Laboratory Chemical Management for Waste Reduction", available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington D.C. 20036, (202)872-4477.

15.0 WASTE MANAGEMENT

15.1 The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management consult "The Waste Management Manual for Laboratory Personnel", available from the American Chemical Society at the address listed in the Section 14.2.

16.0 REFERENCES

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17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

TABLE 1: ESTIMATED INSTRUMENT DETECTION LIMITS

Element	Recommended Analytical Mass	Scanning Mode[1]	Selection Ion Monitoring Mode[2,3]
Aluminum	27	0.05	0.02
Antimony	123	0.08	0.008
Arsenic(3)	75	0.9	0.02
Barium	137	0.5	0.03
Beryllium	9	0.1	0.02
Cadmium	111	0.1	0.02
Chromium	52	0.07	0.04
Cobalt	59	0.03	0.002
Copper	63	0.03	0.004
Lead	206, 207, 208	0.08	0.015
Manganese	55	0.1	0.007
Mercury	202	n.a	0.2
Molybdenum	98	0.1	0.005
Nickel	60	0.2	0.07
Selenium(3)	82	5	1.3
Silver	107	0.05	0.004
Thallium	205	0.09	0.014
Thorium	232	0.03	0.005
Uranium	238	0.02	0.005
Vanadium	51	0.02	0.006
Zinc	66	0.2	0.07

Instrument detection limits (3σ) estimated from seven replicate integrations of the blank (1% v/v nitric acid) following calibration of the instrument with three replicate integrations of a multi-element standard.

[1]Instrument operating conditions and data acquisition mode are given in Table 6.

[2]IDLs determined using state-of-the-art instrumentation (1994). Data for ^{75}As, ^{77}Se, and ^{82}Se were acquired using a dwell time of 4.096 seconds with 1500 area count per sec ^{83}Kr present in argon supply. All other data were acquired using a dwell time of 1.024 seconds per AMU monitored.

TABLE 2: COMMON MOLECULAR ION INTERFERENCES IN ICP-MS

BACKGROUND MOLECULAR IONS		
Molecular Ion	**Mass**	**Element Interference[a]**
NH^+	15	
OH^+	17	
OH_2^+	18	
C_2^+	24	
CN^+	26	
CO^+	28	
N_2^+	28	
N_2H^+	29	
NO^+	30	
NOH^+	31	
O_2^+	32	
O_2H^+	33	
$^{36}ArH^+$	37	
$^{38}ArH^+$	39	
$^{40}ArH^+$	41	
CO_2^+	44	
CO_2H^+	45	Sc
ArC^+, ArO^+	52	Cr
ArN^+	54	Cr
$ArNH^+$	55	Mn
ArO^+	56	
$ArOH^+$	57	
$^{40}Ar^{36}Ar^+$	76	Se
$^{40}Ar^{38}Ar^+$	78	Se
$^{40}Ar^+$	80	Se

[a]method elements or internal standards affected by the molecular ions.

TABLE 2: COMMON MOLECULAR ION INTERFERENCES IN ICP-MS (Cont'd)

MATRIX MOLECULAR IONS

Molecular Ion		Mass	Element Interference[a]
Bromide[12]			
	$^{81}BrH^+$	82	Se
	$^{79}BrO^+$	95	Mo
	$^{81}BrO^+$	97	Mo
	$^{81}BrOH^+$	98	Mo
	$Ar^{81}Br^+$	121	Sb
Chloride			
	$^{35}ClO^+$	51	V
	$^{35}ClOH^+$	52	Cr
	$^{37}ClO^+$	53	Cr
	$^{37}ClOH^+$	54	Cr
	$Ar^{35}Cl^+$	75	As
	$Ar^{37}Cl^+$	77	Se
Sulphate			
	$^{32}SO^+$	48	
	$^{32}SOH^+$	49	
	$^{34}SO^+$	50	V, Cr
	$^{34}SOH^+$	51	V
	SO_2^+, S_2^+	64	Zn
	$Ar^{32}S^+$	72	
	$Ar^{34}S^+$	74	
Phosphate			
	PO^+	47	
	POH^+	48	
	PO_2^+	63	Cu
	ArP^+	71	
Group I, II Metals			
	$ArNa^+$	63	Cu
	ArK^+	79	
	$ArCa^+$	80	

TABLE 2: COMMON MOLECULAR ION INTERFERENCES IN ICP-MS (Cont'd)

MATRIX MOLECULAR IONS		
Molecular Ion	**Mass**	**Element Interference[a]**
Matrix Oxides[*]		
TiO	62-66	Ni, Cu, Zn
ZrO	106-112	Ag, Cd
MoO	108-116	Cd

[*]Oxide interferences will normally be very small and will only impact the method elements when present at relatively high concentrations. Some examples of matrix oxides are listed of which the analyst should be aware. It is recommended that Ti and Zr isotopes are monitored in solid waste samples, which are likely to contain high levels of these elements. Mo is monitored as a method analyte.

TABLE 3: INTERNAL STANDARDS AND LIMITATIONS OF USE

Internal Standard	Mass	Possible Limitation
^{6}Lithium	6	a
Scandium	45	polyatomic ion interference
Yttrium	89	a,b
Rhodium	103	
Indium	115	isobaric interference by Sn
Terbium	159	
Holmium	165	
Lutetium	175	
Bismuth	209	a

a May be present in environmental samples.

b In some instruments Yttrium may form measurable amounts of YO^{+} (105 amu)and YOH^{+} (106 amu). If this is the case, care should be taken in the use of the cadmium elemental correction equation.

Internal standards recommended for use with this method are shown in bold face. Preparation procedures for these are included in Section 7.3.

TABLE 4: RECOMMENDED ANALYTICAL ISOTOPES AND ADDITIONAL MASSES WHICH MUST BE MONITORED

Isotope	Element of Interest
27	Aluminum
121, 123	Antimony
75	Arsenic
135, 137	Barium
9	Beryllium
106, 108, 111, 114	Cadmium
52, 53	Chromium
59	Cobalt
63, 65	Copper
206, 207, 208	Lead
55	Manganese
95, 97, 98	Molybdenum
60, 62	Nickel
77, 82	Selenium
107, 109	Silver
203, 205	Thallium
232	Thorium
238	Uranium
51	Vanadium
66, 67, 68	Zinc
83	Krypton
99	Ruthenium
105	Palladium
118	Tin

NOTE: Isotopes recommended for analytical determination are underlined.

TABLE 5: RECOMMENDED ELEMENTAL EQUATIONS FOR DATA CALCULATIONS

Element	Elemental Equation	Note
Al	(1.000) (^{27}C)	
Sb	(1.000) (^{123}C)	
As	(1.000) (^{75}C)-(3.127) [(^{77}C)-(0.815) (^{82}C)]	(1)
Ba	(1.000) (^{137}C)	
Be	(1.000) (^{9}C)	
Cd	(1.000) (^{111}C)-(1.073) [(^{108}C)-(0.712) (^{106}C)]	(2)
Cr	(1.000) (^{52}C)	(3)
Co	(1.000) (^{59}C)	
Cu	(1.000) (^{63}C)	
Pb	(1.000) (^{206}C)+(1.000) [(^{207}C)+(1.000) (^{208}C)]	(4)
Mn	(1.000) (^{55}C)	
Mo	(1.000) (^{98}C)-(0.146) (^{99}C)	(5)
Ni	(1.000) (^{60}C)	
Se	(1.000) (^{82}C)	(6)
Ag	(1.000) (^{107}C)	
Tl	(1.000) (^{205}C)	
Th	(1.000) (^{232}C)	
U	(1.000) (^{238}C)	
V	(1.000) (^{51}C)-(3.127) [(^{53}C)-(0.113) (^{52}C)]	(7)
Zn	(1.000) (^{66}C)	

TABLE 5: RECOMMENDED ELEMENTAL EQUATIONS FOR DATA CALCULATIONS

Element	Elemental Equation	Note
Bi	(1.000) (^{209}C)	
In	(1.000) (^{209}C)-(0.016) (^{118}C)	(8)
Sc	(1.000) (^{45}C)	
Tb	(1.000) (^{159}C)	
Y	(1.000) (^{89}C)	

C - Calibration blank subtracted counts at specified mass.

(1) - Correction for chloride interference with adjustment for ^{77}Se. ArCl 75/77 ratio may be determined from the reagent blank. Isobaric mass 82 must be from Se only and not BrH^+.

(2) - Correction for MoO interference. Isobaric mass 106 must be from Cd only not ZrO^+. An additional isobaric elemental correction should be made if palladium is present.

(3) - In 0.4% v/v HCl, the background from ClOH will normally be small. However the contribution may be estimated from the reagent blank. Isobaric mass must be from Cr only not ArC^+.

(4) - Allowance for isotopic variability of lead isotopes.

(5) - Isobaric elemental correction for ruthenium.

(6) - Some argon supplies contain krypton as an impurity. Selenium is corrected for ^{82}Kr by background subtraction.

(7) - Correction for chloride interference with adjustment for ^{53}Cr. ClO 51/53 ratio may be determined from the reagent blank. Isobaric mass 52 must be from Cr only not ArC^+.

(8) - Isobaric elemental correction for tin.

TABLE 6: INSTRUMENT OPERATING CONDITIONS FOR PRECISION AND RECOVERY DATA[1]

Instrument	VG PlasmaQuad Type I
Plasma foward power	1.35 kW
Coolant flow rate	13.5 L/min.
Auxillary flow rate	0.6 L/min.
Nebulizer flow rate	0.78 L/min.
Solution uptake rate	0.6 mL/min.
Spray chamber temperature	15°C
Data Acquistion	
Detector mode	Pulse counting
Replicate integrations	3
Mass range	8-240 amu
Dwell time	320 µs
Number of MCA channels	2048
Number of scan sweeps	85
Total acquisition time	3 minutes per sample

[1]The described instrument and operating conditions were used to determine the scanning mode MDL data listed in Table 7 and the precision and recovery data given in Tables 9 and 10.

TABLE 7: METHOD DETECTION LIMITS

	Scanning Mode[1] Total Recoverable		Selection Ion Monitoring Mode[2] Total Recoverable	Direct Analysis[3]
AMU Element	Aqueous µg/L	Solids mg/kg	Aqueous µg/L	Aqueous µg/L
27 Al	1.0	0.4	1.7	0.04
123 Sb	0.4	0.2	0.04	0.02
75 As	1.4	0.6	0.4	0.1
137 Ba	0.8	0.4	0.04	0.04
9 Be	0.3	0.1	0.02	0.03
111 Cd	0.5	0.2	0.03	0.03
52 Cr	0.9	0.4	0.08	0.08
59 Co	0.09	0.04	0.004	0.003
63 Cu	0.5	0.2	0.02	0.01
206,207,208 Pb	0.6	0.3	0.05	0.02
55 Mn	0.1	0.05	0.02	0.04
202 Hg	n.a.	n.a.	n.a	0.2
98 Mo	0.3	0.1	0.01	0.01
60 Ni	0.5	0.2	0.06	0.03
82 Se	7.9	3.2	2.1	0.5
107 Ag	0.1	0.05	0.005	0.005
205 Tl	0.3	0.1	0.02	0.01
232 Th	0.1	0.05	0.02	0.01
238 U	0.1	0.05	0.01	0.01
51 V	2.5	1.0	0.9	0.05
66 Zn	1.8	0.7	0.1	0.2

[1]Data acquisition mode given in Table 6. Total recoverable MDL concentrations are computed for original matrix with allowance for sample dilution during preparation. Listed MDLs for solids calculated from determined aqueous MDLs.

[2]MDLs determined using state-of-the-art instrumentation (1994). Data for ^{75}As, ^{77}Se, and ^{82}Se were acquired using a dwell time of 4.096 seconds with 1500 area count per seconds ^{83}Kr present in argon supply. All other data were acquired using a dwell time of 1.024 seconds per AMU monitored.

[3]MDLs were determined from analysis of seven undigested aqueous sample aliquots.

n.a. - Not applicable. Total recoverable digestion not suitable for organo-mercury compounds.

TABLE 8: ACCEPTANCE LIMITS FOR QC CHECK SAMPLE

METHOD PERFORMANCE (µg/L)[1]

Element	QC Check Sample Conc.	Average Recovery	Standard Deviation[2] (S_r)	Acceptance Limits[3] µg/L
Aluminum	100	100.4	5.49	84-117
Antimony	100	99.9	2.40	93-107
Arsenic	100	101.6	3.66	91-113
Barium	100	99.7	2.64	92-108
Beryllium	100	105.9	4.13	88-112[4]
Cadmium	100	100.8	2.32	94-108
Chromium	100	102.3	3.91	91-114
Cobalt	100	97.7	2.66	90-106
Copper	100	100.3	2.11	94-107
Lead	100	104.0	3.42	94-114
Manganese	100	98.3	2.71	90-106
Molybdenum	100	101.0	2.21	94-108
Nickel	100	100.1	2.10	94-106
Selenium	100	103.5	5.67	86-121
Silver	100	101.1	3.29	91-111[5]
Thallium	100	98.5	2.79	90-107
Thorium	100	101.4	2.60	94-109
Uranium	100	102.6	2.82	94-111
Vanadium	100	100.3	3.26	90-110
Zinc	100	105.1	4.57	91-119

[1]Method performance characteristics calculated using regression equations from collaborative study, Reference 11.

[2]Single-analyst standard deviation, S_r.

[3]Acceptance limits calculated as average recovery ± three standard deviations.

[4]Acceptance limits centered at 100% recovery.

[5]Statistics estimated from summary statistics at 48 and 64 µg/L.

TABLE 9: PRECISION AND RECOVERY DATA IN AQUEOUS MATRICES

DRINKING WATER

Element	Sample Conc. µg/L	Low Spike µg/L	Average Recovery R (%)	S (R)	RPD	High Spike µg/L	Average Recovery R (%)	S (R)	RPD
Al	175	50	115.8	5.9	0.4	200	102.7	1.6	1.1
Sb	<0.4	10	99.1	0.7	2.0	100	100.8	0.7	2.0
As	<1.4	50	99.7	0.8	2.2	200	102.5	1.1	2.9
Ba	43.8	50	94.8	3.9	5.8	200	95.6	0.8	1.7
Be	<0.3	10	113.5	0.4	0.9	100	111.0	0.7	1.8
Cd	<0.5	10	97.0	2.8	8.3	100	101.5	0.4	1.0
Cr	<0.9	10	111.0	3.5	9.0	100	99.5	0.1	0.2
Co	0.11	10	94.4	0.4	1.1	100	93.6	0.5	1.4
Cu	3.6	10	101.8	8.8	17.4	100	91.6	0.3	0.3
Pb	0.87	10	97.8	2.0	2.8	100	99.0	0.8	2.2
Mn	0.96	10	96.9	1.8	4.7	100	95.8	0.6	1.8
Mo	1.9	10	99.4	1.6	3.4	100	98.6	0.4	1.0
Ni	1.9	10	100.2	5.7	13.5	100	95.2	0.5	1.3
Se	<7.9	50	99.0	1.8	5.3	200	93.5	3.5	10.7
Ag	<0.1	50	100.7	1.5	4.2	200	99.0	0.4	1.0
Tl	<0.3	10	97.5	0.4	1.0	100	98.5	1.7	4.9
Th	<0.1	10	109.0	0.7	1.8	100	106.0	1.4	3.8
U	0.23	10	110.7	1.4	3.5	100	107.8	0.7	1.9
V	<2.5	50	101.4	0.1	0.4	200	97.5	0.7	2.1
Zn	5.2	50	103.4	3.3	7.7	200	96.4	0.5	1.0

S (R) Standard deviation of percent recovery.
RPD Relative percent difference between duplicate spike determinations.
< Sample concentration below established method detection limit.

TABLE 9: PRECISION AND RECOVERY DATA IN AQUEOUS MATRICES (Cont'd)

WELL WATER

Element	Sample Conc. µg/L	Low Spike µg/L	Average Recovery R (%)	S (R)	RPD	High Spike µg/L	Average Recovery R (%)	S (R)	RPD
Al	34.3	50	100.1	3.9	0.8	200	102.6	1.1	1.3
Sb	0.46	10	98.4	0.9	1.9	100	102.5	0.7	1.9
As	<1.4	50	110.0	6.4	16.4	200	101.3	0.2	0.5
Ba	106	50	95.4	3.9	3.3	200	104.9	1.0	1.6
Be	<0.3	10	104.5	0.4	1.0	100	101.4	1.2	3.3
Cd	106	10	88.6	1.7	3.8	100	98.6	0.6	1.6
Cr	<0.9	10	111.0	0.0	0.0	100	103.5	0.4	1.0
Co	2.4	10	100.6	1.0	1.6	100	104.1	0.4	0.9
Cu	37.4	10	104.3	5.1	1.5	100	100.6	0.8	1.5
Pb	3.5	10	95.2	2.5	1.5	100	99.5	1.4	3.9
Mn	2770	10	*	*	1.8	100	*	*	0.7
Mo	2.1	10	103.8	1.1	1.6	100	102.9	0.7	1.9
Ni	11.4	10	116.5	6.3	6.5	100	99.6	0.3	0.0
Se	<7.9	50	127.3	8.4	18.7	200	101.3	0.2	0.5
Ag	<0.1	50	99.2	0.4	1.0	200	101.5	1.4	3.9
Tl	<0.3	10	93.9	0.1	0.0	100	100.4	1.8	5.0
Th	<0.1	10	103.0	0.7	1.9	100	104.5	1.8	4.8
U	1.8	10	106.0	1.1	1.6	100	109.7	2.5	6.3
V	<2.5	50	105.3	0.8	2.1	200	105.8	0.2	0.5
Zn	554	50	*	*	1.2	200	102.1	5.5	3.2

S (R) Standard deviation of percent recovery.
RPD Relative percent difference between duplicate spike determinations.
< Sample concentration below established method detection limit.
* Spike concentration <10% of sample background concentration.

TABLE 9: PRECISION AND RECOVERY DATA IN AQUEOUS MATRICES (Cont'd)

POND WATER

Element	Sample Conc. µg/L	Low Spike µg/L	Average Recovery R (%)	S (R)	RPD	High Spike µg/L	Average Recovery R (%)	S (R)	RPD
Al	610	50	*	*	1.7	200	78.2	9.2	5.5
Sb	<0.4	10	101.1	1.1	2.9	100	101.5	3.0	8.4
As	<1.4	50	100.8	2.0	5.6	200	96.8	0.9	2.6
Ba	28.7	50	102.1	1.8	2.4	200	102.9	3.7	9.0
Be	<0.3	10	109.1	0.4	0.9	100	114.4	3.9	9.6
Cd	<0.5	10	106.6	3.2	8.3	100	105.8	2.8	7.6
Cr	2.0	10	107.0	1.0	1.6	100	100.0	1.4	3.9
Co	0.79	10	101.6	1.1	2.7	100	101.7	1.8	4.9
Cu	5.4	10	107.5	1.4	1.9	100	98.1	2.5	6.8
Pb	1.9	10	108.4	1.5	3.2	100	106.1	0.0	0.0
Mn	617	10	*	*	1.1	100	139.0	11.1	4.0
Mo	0.98	10	104.2	1.4	3.5	100	104.0	2.1	5.7
Ni	2.5	10	102.0	2.3	4.7	100	102.5	2.1	5.7
Se	<7.9	50	102.7	5.6	15.4	200	105.5	1.4	3.8
Ag	0.12	50	102.5	0.8	2.1	200	105.2	2.7	7.1
Tl	<0.3	10	108.5	3.2	8.3	100	105.0	2.8	7.6
Th	0.19	10	93.1	3.5	10.5	100	93.9	1.6	4.8
U	0.30	10	107.0	2.8	7.3	100	107.2	1.8	4.7
V	3.5	50	96.1	5.2	14.2	200	101.5	0.2	0.5
Zn	6.8	50	99.8	1.7	3.7	200	100.1	2.8	7.7

S (R) Standard deviation of percent recovery.
RPD Relative percent difference between duplicate spike determinations.
< Sample concentration below established method detection limit.
* Spike concentration <10% of sample background concentration.

TABLE 9: PRECISION AND RECOVERY DATA IN AQUEOUS MATRICES (Cont'd)

SEWAGE TREATMENT PRIMARY EFFLUENT

Element	Sample Conc. µg/L	Low Spike µg/L	Average Recovery R (%)	S (R)	RPD	High Spike µg/L	Average Recovery R (%)	S (R)	RPD
Al	1150	50	*	*	3.5	200	100.0	13.8	1.5
Sb	1.5	10	95.7	0.4	0.9	100	104.5	0.7	1.9
As	<1.4	50	104.2	4.5	12.3	200	101.5	0.7	2.0
Ba	202	50	79.2	9.9	2.5	200	108.6	4.6	5.5
Be	<0.3	10	110.5	1.8	4.5	100	106.4	0.4	0.9
Cd	9.2	10	101.2	1.3	0.0	100	102.3	0.4	0.9
Cr	128	10	*	*	1.5	100	102.1	1.7	0.4
Co	13.4	10	95.1	2.7	2.2	100	99.1	1.1	2.7
Cu	171	10	*	*	2.4	100	105.2	7.1	0.7
Pb	17.8	10	95.7	3.8	1.1	100	102.7	1.1	2.5
Mn	199	10	*	*	1.5	100	103.4	2.1	0.7
Mo	136	10	*	*	1.4	100	105.7	2.4	2.1
Ni	84.0	10	88.4	16.3	4.1	100	98.0	0.9	0.0
Se	<7.9	50	112.0	10.9	27.5	200	108.8	3.0	7.8
Ag	10.9	50	97.1	0.7	1.5	200	102.6	1.4	3.7
Tl	<0.3	10	97.5	0.4	1.0	100	102.0	0.0	0.0
Th	0.11	10	15.4	1.8	30.3	100	29.3	0.8	8.2
U	0.71	10	109.4	1.8	4.3	100	109.3	0.7	1.8
V	<2.5	50	90.9	0.9	0.6	200	99.4	2.1	6.0
Zn	163	50	85.8	3.3	0.5	200	102.0	1.5	1.9

S (R) Standard deviation of percent recovery.
RPD Relative percent difference between duplicate spike determinations.
< Sample concentration below established method detection limit.
* Spike concentration <10% of sample background concentration.

TABLE 9: PRECISION AND RECOVERY DATA IN AQUEOUS MATRICES (Cont'd)

INDUSTRIAL EFFLUENT

Element	Sample Conc. µg/L	Low Spike µg/L	Average Recovery R (%)	S (R)	RPD	High Spike µg/L	Average Recovery R (%)	S (R)	RPD
Al	44.7	50	98.8	8.7	5.7	200	90.4	2.1	2.2
Sb	2990	10	*	*	0.3	100	*	*	0.0
As	<1.4	50	75.1	1.8	6.7	200	75.0	0.0	0.0
Ba	100	50	96.7	5.5	3.4	200	102.9	1.1	0.7
Be	<0.3	10	103.5	1.8	4.8	100	100.0	0.0	0.0
Cd	10.1	10	106.5	4.4	2.4	100	97.4	1.1	2.8
Cr	171	10	*	*	0.0	100	127.7	2.4	1.7
Co	1.3	10	90.5	3.2	8.7	100	90.5	0.4	1.3
Cu	101	10	*	*	0.9	100	92.5	2.0	1.6
Pb	294	10	*	*	2.6	100	108.4	2.1	0.0
Mn	154	10	*	*	2.8	100	103.6	3.7	1.6
Mo	1370	10	*	*	1.4	100	*	*	0.7
Ni	17.3	10	107.4	7.4	5.0	100	88.2	0.7	1.0
Se	15.0	50	129.5	9.3	15.1	200	118.3	1.9	3.6
Ag	<0.1	50	91.8	0.6	1.7	200	87.0	4.9	16.1
Tl	<0.3	10	90.5	1.8	5.5	100	98.3	1.0	2.8
Th	0.29	10	109.6	1.2	2.7	100	108.7	0.0	0.0
U	0.17	10	104.8	2.5	6.6	100	109.3	0.4	0.9
V	<2.5	50	74.9	0.1	0.3	200	72.0	0.0	0.0
Zn	43.4	50	85.0	4.0	0.6	200	97.6	1.0	0.4

S (R) Standard deviation of percent recovery.
RPD Relative percent difference between duplicate spike determinations.
< Sample concentration below established method detection limit.
* Spike concentration <10% of sample background concentration.

TABLE 10: PRECISION AND RECOVERY DATA IN SOLID MATRICES

EPA HAZARDOUS SOIL #884

Element	Sample Conc. (mg/kg)	Low+ Spike (mg/kg)	Average Recovery R (%)	S (R)	RPD	High+ Spike (mg/kg)	Average Recovery R (%)	S (R)	RPD
Al	5170	20	*	*	–	100	*	*	–
Sb	5.4	20	69.8	2.5	4.7	100	70.4	1.8	6.5
As	8.8	20	104.7	5.4	9.1	100	102.2	2.2	5.4
Ba	113	20	54.9	63.6	18.6	100	91.0	9.8	0.5
Be	0.6	20	100.1	0.6	1.5	100	102.9	0.4	1.0
Cd	1.8	20	97.3	1.0	1.4	100	101.7	0.4	1.0
Cr	83.5	20	86.7	16.1	8.3	100	105.5	1.3	0.0
Co	7.1	20	98.8	1.2	1.9	100	102.9	0.7	1.8
Cu	115	20	86.3	13.8	3.4	100	151.7	4.2	4.6
Pb	152	20	85.0	45.0	13.9	100	85.2	25.7	23.7
Mn	370	20	*	*	12.7	100	95.2	10.4	2.2
Mo	4.8	20	95.4	1.5	2.9	100	102.3	0.7	2.0
Ni	19.2	20	101.7	3.8	1.0	100	100.7	0.8	0.8
Se	<3.2	20	79.5	7.4	26.4	100	94.8	9.4	26.5
Ag	1.1	20	96.1	0.6	0.5	100	97.9	0.8	2.3
Tl	0.24	20	94.3	1.1	3.1	100	76.0	1.0	2.9
Th	1.0	20	69.8	0.6	1.3	100	102.9	2.2	7.9
U	1.1	20	100.1	0.2	0.0	100	106.7	0.0	0.0
V	17.8	20	109.2	4.2	2.3	100	113.4	1.3	2.4
Zn	128	20	87.0	27.7	5.5	100		12.9	14.1

S (R) Standard deviation of percent recovery.
RPD Relative percent difference between duplicate spike determinations.
< Sample concentration below established method detection limit.
* Spike concentration <10% of sample background concentration.
– Not determined.
+ Equivalent.

TABLE 10: PRECISION AND RECOVERY DATA IN SOLID MATRICES

NBS 1645 RIVER SEDIMENT

Element	Sample Conc. (mg/kg)	Low[+] Spike (mg/kg)	Average Recovery R (%)	S (R)	RPD	High[+] Spike (mg/kg)	Average Recovery R (%)	S (R)	RPD
Al	5060	20	*	*	–	100	*	*	–
Sb	21.8	20	73.9	6.5	9.3	100	81.2	1.5	3.9
As	67.2	20	104.3	13.0	7.6	100	107.3	2.1	2.9
Ba	54.4	20	105.6	4.9	2.8	100	98.6	2.2	3.9
Be	0.59	20	88.8	0.2	0.5	100	87.9	0.1	0.2
Cd	8.3	20	92.9	0.4	0.0	100	95.7	1.4	3.9
Cr	29100	20	*	*	–	100	*	*	–
Co	7.9	20	97.6	1.3	2.6	100	103.1	0.0	0.0
Cu	112	20	121.0	9.1	1.5	100	105.2	2.2	1.8
Pb	742	20	*	*	–	100	–	–	–
Mn	717	20	*	*	–	100	–	–	–
Mo	17.1	20	89.8	8.1	12.0	100	98.4	0.7	0.9
Ni	41.8	20	103.7	6.5	4.8	100	102.2	0.8	0.0
Se	<3.2	20	108.3	14.3	37.4	100	93.9	5.0	15.1
Ag	1.8	20	94.8	1.6	4.3	100	96.2	0.7	1.9
Tl	1.2	20	91.2	1.3	3.6	100	94.4	0.4	1.3
Th	0.90	20	91.3	0.9	2.6	100	92.3	0.9	2.8
U	0.79	20	95.6	1.8	5.0	100	98.5	1.2	3.5
V	21.8	20	91.8	4.6	5.7	100	100.7	0.6	0.8
Zn	1780	20	*	*	–	100	*	*	–

S (R) Standard deviation of percent recovery.
RPD Relative percent difference between duplicate spike determinations.
< Sample concentration below established method detection limit.
* Spike concentration <10% of sample background concentration.
– Not determined.
[+] Equivalent.

TABLE 10: PRECISION AND RECOVERY DATA IN SOLID MATRICES

EPA ELECTROPLATING SLUDGE #286

Element	Sample Conc. (mg/kg)	Low+ Spike (mg/kg)	Average Recovery R (%)	S (R)	RPD	High+ Spike (mg/kg)	Average Recovery R (%)	S (R)	RPD
Al	5110	20	*	*	–	100	*	*	–
Sb	8.4	20	55.4	1.5	4.1	100	61.0	0.2	0.9
As	41.8	20	91.0	2.3	1.7	100	94.2	0.8	1.5
Ba	27.3	20	1.8	7.1	8.3	100	0	1.5	10.0
Be	0.25	20	92.0	0.9	2.7	100	93.4	0.3	0.9
Cd	112	20	85.0	5.2	1.6	100	88.5	0.8	0.5
Cr	7980	20	*	*	–	100	*	*	–
Co	4.1	20	89.2	1.8	4.6	100	88.7	1.5	4.6
Cu	740	20	*	*	6.0	100	61.7	20.4	5.4
Pb	1480	20	*	*	–	100	*	*	–
Mn	295	20	*	*	–	100	–	–	–
Mo	13.3	20	82.9	1.2	1.3	100	89.2	0.4	1.0
Ni	450	20	*	*	6.8	100	83.0	10.0	4.5
Se	3.5	20	89.7	3.7	4.2	100	91.0	6.0	18.0
Ag	5.9	20	89.8	2.1	4.6	100	85.1	0.4	1.1
Tl	1.9	20	96.9	0.9	2.4	100	98.9	0.9	2.4
Th	3.6	20	91.5	1.3	3.2	100	97.4	0.7	2.0
U	2.4	20	107.7	2.0	4.6	100	109.6	0.7	1.8
V	21.1	20	105.6	1.8	2.1	100	97.4	1.1	2.5
Zn	13300	20	*	*	–	100	*	*	–

S (R) Standard deviation of percent recovery.
RPD Relative percent difference between duplicate spike determinations.
< Sample concentration below established method detection limit.
* Spike concentration <10% of sample background concentration.
– Not determined.
+ Equivalent.

TABLE 11: PRIMARY DRINKING WATER CONTAMINANTS
PRECISION AND RECOVERY DATA

Analyte	Regional Sample Background Concentration, µg/L (IV)	(VI)	(X)	Average Mean[1] % Recovery	S (R)
Antimony	0.16	0.07	0.03	114%	1.9
Arsenic	< MDL	2.4	1.0	93	8.5
Barium	4.6	280	14.3	(*)	–
Beryllium	< MDL	< MDL	< MDL	100%	8.2
Cadmium	0.05	0.05	0.03	81	4.0
Chromium	0.71	5.1	0.10	94	2.5
Copper	208	130	14.3	(*)	–
Lead	1.2	1.2	2.5	91	2.6
Mercury	< MDL	0.23	< MDL	86	11.4
Nickel	1.7	3.6	0.52	101%	11.5
Selenium	< MDL	4.3	< MDL	98	8.4
Thallium	< MDL	0.01	< MDL	100	1.4

[1]The three regional waters were fortified with 1.0 µg/L of all analytes listed, except selenium, which was fortified to 20 µg/L.

(*) Recovery of barium and copper was not calculated because the analyte addition was <20% the sample background concentration in all waters. (Recovery calculations are not required if the concentration of the analyte added is less than 30% of the sample background concentration. Section 9.4.3).

S (R) Standard deviation of the mean percent recoveries.

TABLE 12: SUMMARY STATISTICS AND DESCRIPTIVE EQUATIONS FOR THE 20 ANALYTES TESTED IN THE COLLABORATIVE STUDY

		Reagent Water				Finished Drinking Water				Ground Water			
Analyte	C[a]	$\bar{X}$[b]	S_R	S_r	Regr. Equations	$\bar{X}$	S_R	S_r	Regr. Equations	$\bar{X}$	S_R	S_r	Regr. Equations
Aluminum	8.00	10.01	2.33	1.74	$\bar{X} = 0.992C + 1.19$	11.18	9.02	6.34	$\bar{X} = 0.954C + 2.38$	9.86	7.10	2.70	$\bar{X} = 0.946C + 2.20$
	12.00	10.98	5.16		$S_R = 0.056\bar{X} + 2.59$[c]	11.02	3.02		$S_R = 7.70$[d]	13.40	10.27		$S_R = 0.169\bar{X} + 6.22$[c]
	56.00	59.13	5.55	4.19	$S_r = 0.042X + 1.27$	56.97	7.14	6.18	$S_r = 0.013\bar{X} + 6.17$	51.75	10.78	16.92	$S_r = 0.172\bar{X} + 0.75$[c]
	80.00	82.59	4.92			82.73	8.01			82.83	33.37		
	160.00	158.95	11.82	8.90		159.89	11.94	10.59		155.40	15.39	19.27	
	200.00	200.89	8.61			189.98	12.97			189.64	31.46		
Antimony	2.80	2.75	0.27	0.27	$\bar{X} = 0.999C + 0.04$	2.73	0.29	0.17	$\bar{X} = 0.983C + 0.03$	2.82	0.19	0.22	$\bar{X} = 1.003C + 0.01$
	4.00	4.22	0.46		$S_R = 0.013\bar{X} + 0.61$[e]	4.10	0.47		$S_R = 0.049\bar{X} + 0.19$	4.02	0.35		$S_R = 0.059\bar{X} + 0.04$
	20.00	19.76	1.09	0.85	$S_r = 0.022X + 0.20$	19.17	1.37	0.66	$S_r = 0.026\bar{X} + 0.08$	20.12	0.82	0.97	$S_r = 0.058\bar{X} + 0.02$
	28.00	27.48	1.38			26.48	1.72			27.77	1.38		
	80.00	82.52	2.24	1.76		83.43	2.05	2.46		80.34	9.14	6.80	
	100.00	98.06	1.34			97.19	5.31			101.09	2.89		
Arsenic	8.00	8.64	3.01	3.02	$\bar{X} = 1.013C + 0.50$	9.00	3.13	1.96	$\bar{X} = 0.993C + 0.57$	10.40	5.17	4.90	$\bar{X} = 0.949C + 0.91$
	12.00	12.58	3.18		$S_R = 0.031\bar{X} + 2.74$	11.37	1.77		$S_R = 0.018\bar{X} + 2.55$	7.85	4.62		$S_R = 0.048\bar{X} + 4.52$
	56.00	55.44	4.64	3.51	$S_r = 0.007X + 2.95$	53.77	4.12	4.07	$S_r = 0.031X + 1.65$	53.25	3.49	7.88	$S_r = 0.059X + 4.29$
	80.00	85.15	2.54			87.72	4.14			83.60	12.46		
	160.00	161.80	11.15	3.96		157.56	4.83	6.30		159.86	11.67	14.94	
	200.00	201.52	10.81			197.99	10.66			194.41	18.24		
Barium	8.01	7.58	0.50	0.48	$\bar{X} = 1.001C - 0.36$	8.21	1.21	1.11	$\bar{X} = 0.995C + 0.37$	8.04	2.60	2.24	$\bar{X} = 1.055C - 0.21$
	12.00	11.81	1.05		$S_R = 0.039\bar{X} + 0.31$	12.56	1.79		$S_R = 0.045\bar{X} + 0.97$[c]	12.85	1.45		$S_R = 0.020\bar{X} + 2.05$
	48.00	47.32	1.60	1.82	$S_r = 0.024X + 0.25$	49.13	3.72	3.77	$S_r = 0.040\bar{X} + 0.72$[c]	50.12	2.98	2.19	$S_r = 0.014\bar{X} + 2.08$
	64.00	65.52	2.90			65.30	4.16			69.53	2.66		
	160.00	157.09	6.53	4.07		155.25	7.82	5.67		164.44	8.81	6.61	
	200.00	198.53	8.28			196.52	5.70			208.32	9.22		
Beryllium	2.80	3.31	0.81	0.26	$\bar{X} = 1.056C + 0.32$	3.15	0.47	0.31	$\bar{X} = 1.055C + 0.20$	3.02	0.46	0.22	$\bar{X} = 1.049C + 0.08$
	4.00	4.45	0.73		$S_R = 0.067\bar{X} + 0.55$	4.45	0.51		$S_R = 0.057\bar{X} + 0.28$	4.27	0.44		$S_R = 0.084\bar{X} + 0.16$
	20.00	22.38	2.76	1.00	$S_r = 0.038X + 0.11$	21.27	1.23	0.63	$S_r = 0.016X + 0.25$	21.55	1.72	1.10	$S_r = 0.043X + 0.06$
	28.00	30.02	2.86			29.57	1.67			29.24	2.09		
	80.00	84.18	4.79	4.02		87.59	6.89	1.88		84.23	9.05	4.32	
	100.00	102.88	5.90			102.64	6.27			103.39	10.17		
Cadmium	4.00	4.01	0.34	0.20	$\bar{X} = 1.007C + 0.07$	4.11	0.88	0.71	$\bar{X} = 0.985C + 0.10$	3.98	0.48	0.14	$\bar{X} = 0.944C + 0.11$
	6.00	6.32	0.49		$S_R = 0.041\bar{X} + 0.19$	5.87	0.58		$S_R = 0.031\bar{X} + 0.65$	5.62	0.73		$S_R = 0.017\bar{X} + 1.09$[e]
	20.00	19.81	1.12	0.86	$S_r = 0.022X + 0.10$[c]	19.57	1.45	1.26	$S_r = 0.021\bar{X} + 0.61$	18.15	1.73	0.88	$S_r = 0.029\bar{X} + 0.01$
	28.00	28.33	0.94			27.68	1.27			26.86	2.59		
	80.00	81.28	4.91	1.33		80.62	4.45	2.02		77.83	3.05	1.88	
	100.00	100.11	3.24			98.15	3.60			95.31	2.04		

TABLE 12: SUMMARY STATISTICS AND DESCRIPTIVE EQUATIONS FOR THE 20 ANALYTES TESTED IN THE COLLABORATIVE STUDY

		Reagent Water				Finished Drinking Water				Ground Water			
Analyte	C[a]	$\bar{X}$[b]	S_R	S_r	Regr. Equations	$\bar{X}$	S_R	S_r	Regr. Equations	$\bar{X}$	S_R	S_r	Regr. Equations
Chromium	8.00	8.27	0.32	1.54	$\bar{X} = 1.017\bar{C} + 0.62$	9.46	2.34	2.08	$\bar{X} = 0.990\bar{C} + 1.45$	8.98	1.47	0.37	$\bar{X} = 1.026\bar{C} + 0.89$
	12.00	13.88	3.10		$S_R = 0.066\bar{X} + 0.48$	13.10	2.39		$S_R = 0.015\bar{X} + 2.19$	13.42	1.13		$S_R = 0.067\bar{X}$ 0.68
	56.00	57.86	4.03	2.68	$S_r = 0.026\bar{X} + 1.25$	56.04	2.24	1.29	$S_r = 2.18$[d]	59.35	5.99	5.42	$S_r = 0.068\bar{X} - 0.37$
	80.00	84.73	2.65			84.38	3.18			83.90	5.70		
	160.00	157.66	13.62	6.97		158.24	5.12	3.16		164.58	14.11	9.80	
	200.00	197.43	9.47			196.72	7.47			199.88	11.19		
Cobalt	0.80	0.88	0.10	0.05	$\bar{X} = 0.977\bar{C} + 0.01$	0.92	0.45	0.31	$\bar{X} = 0.964\bar{C} + 0.06$	0.85	0.13	0.09	$\bar{X} = 0.989\bar{C} - 0.01$
	1.21	0.98	0.04		$S_R = 0.028\bar{X} + 0.06$	1.02	0.10		$S_R = 0.019\bar{X} + 0.32$	1.04	0.18		$S_R = 0.057\bar{X} + 0.09$
	20.10	20.77	0.74	0.67	$S_r = 0.027\bar{X} + 0.02$	20.45	0.91	0.53	$S_r = 0.014\bar{X} + 0.30$	20.81	1.11	1.12	$S_r = 0.012\bar{X} + 0.40$[e]
	28.20	27.75	0.96			27.29	1.22			28.07	2.16		
	80.50	78.59	2.29	2.31		78.04	3.72	1.84		79.26	4.66	1.34	
	101.00	98.79	2.94			97.62	4.62			99.41	4.22		
Copper	4.00	3.88	0.73	0.59	$\bar{X} = 1.003\bar{C} - 0.05$	3.33	0.85	0.99	$\bar{X} = 0.976\bar{C} - 0.38$	3.86	1.40	0.71	$\bar{X} = 0.977\bar{C} - 0.01$
	6.00	6.14	1.00		$S_R = 0.037\bar{X} + 0.64$	5.95	1.78		$S_R = 0.063\bar{X} + 0.86$	5.96	0.95		$S_R = 0.073\bar{X} + 0.92$
	20.00	20.07	1.08	0.92	$S_r = 0.016\bar{X} + 0.51$	18.90	1.64	1.51	$S_r = 0.029\bar{X} + 0.86$	18.97	1.68	2.32	$S_r = 0.077\bar{X} + 0.35$
	28.00	27.97	1.94			27.21	2.76			27.44	2.58		
	80.00	79.80	3.22	1.91		76.64	5.30	3.42		79.30	9.05	6.54	
	100.00	99.57	4.42			96.17	5.64			97.54	11.16		
Lead	4.00	4.00	1.57	1.62	$\bar{X} = 1.043\bar{C} - 0.31$	3.44	1.15	1.18	$\bar{X} = 1.032\bar{C} - 0.30$	4.20	1.13	1.76	$\bar{X} = 1.012\bar{C} + 0.15$
	6.00	5.56	2.00		$S_R = 0.064\bar{X} + 1.43$[c]	6.84	1.10		$S_R = 0.015\bar{X} + 1.06$	6.27	2.38		$S_R = 0.048\bar{X} + 1.27$
	20.00	20.54	2.91	4.36	$S_r = 3.42$[d]	20.18	1.20	1.44	$S_r = 0.011\bar{X} + 1.13$	19.57	2.72	0.88	$S_r = 1.78$[d]
	28.00	30.90	4.58			28.08	1.57			28.55	1.73		
	80.00	80.57	3.13	4.29		80.92	2.30	2.07		82.47	4.38	2.69	
	100.00	102.93	6.62			101.60	3.23			102.47	3.58		
Manganese	0.80	0.86	0.15	0.09	$\bar{X} = 0.983\bar{C} + 0.02$	0.96	0.32	0.42	$\bar{X} = 0.989\bar{C} + 0.10$	0.64	0.22	0.17	$\bar{X} = 0.954\bar{C} - 0.16$
	1.20	1.09	0.12		$S_R = 0.026\bar{X} + 0.11$	1.13	0.38		$S_R = 0.047\bar{X} + 0.29$	0.90	0.21		$S_R = 0.103\bar{X} + 0.14$
	20.00	20.43	0.89	0.72	$S_r = 0.027\bar{X} + 0.06$	21.06	1.32	0.96	$S_r = 0.021\bar{X} + 0.40$	19.61	2.60	2.62	$S_r = 0.025\bar{X} + 0.09$[e]
	28.00	27.53	0.41			27.60	1.47			25.65	4.10		
	80.00	79.00	3.16	2.38		79.57	4.18	2.01		77.38	6.13	2.90	
	100.00	97.60	2.51			97.97	4.10			95.86	6.74		

TABLE 12: SUMMARY STATISTICS AND DESCRIPTIVE EQUATIONS FOR THE 20 ANALYTES TESTED IN THE COLLABORATIVE STUDY

		Reagent Water				Finished Drinking Water				Ground Water			
Analyte	C[a]	$\bar{X}$[b]	S_R	S_r	Regr. Equations	$\bar{X}$	S_R	S_r	Regr. Equations	$\bar{X}$	S_R	S_r	Regr. Equations
Molybdenum	2.80	2.63	0.32	0.16	$\bar{X} = 1.012C - 0.20$	2.80	0.20	0.32	$\bar{X} = 1.013C - 0.07$	3.00	0.47	0.42	$\bar{X} = 1.032C - 0.09$
	4.00	3.85	0.31		$S_R = 0.032\bar{X} + 0.22$	3.95	0.47		$S_R = 0.037\bar{X} + 0.17$	3.60	0.90		$S_R = 0.55\bar{X} + 0.43$
	20.00	19.75	0.64	0.64	$S_r = 0.021\bar{X} + 0.09$	19.78	0.60	1.16	$S_r = 0.035\bar{X} + 0.20$	20.69	1.37	1.11	$S_r = 0.042\bar{X} + 0.27$
	28.00	27.87	1.07			27.87	1.51			28.80	2.01		
	80.00	83.07	3.07	1.78		85.65	3.50	3.07		84.26	4.13	4.81	
	100.00	100.08	4.32			99.06	2.89			103.57	6.10		
Nickel	4.00	4.02	0.41	0.50	$\bar{X} = 1.000C + 0.12$	3.66	0.53	1.03	$\bar{X} = 0.953C - 0.19$	4.81	2.06	2.82	$\bar{X} = 1.022C + 0.66$
	6.00	6.36	0.91		$S_R = 0.051\bar{X} + 0.31$	5.44	1.32		$S_R = 0.046\bar{X} + 0.56$	6.67	3.66		$S_R = 0.091\bar{X} + 2.03$
	20.00	19.93	1.30	0.63	$S_r = 0.017\bar{X} + 0.40$	18.42	0.87	1.11	$S_r = 0.023\bar{X} + 0.91$	20.58	3.71	2.37	$S_r = 0.008\bar{X} + 2.75$[c]
	28.00	28.02	1.25			27.09	1.68			30.73	3.75		
	80.00	79.29	2.95	2.55		75.84	4.40	3.94		82.71	9.49	5.42	
	100.00	100.87	7.20			95.83	4.41			101.00	9.89		
Selenium	32.00	33.54	4.63	1.57	$\bar{X} = 1.036C - 0.06$	32.57	4.37	3.65	$\bar{X} = 1.022C + 0.14$	32.46	4.95	3.24	$\bar{X} = 1.045C - 0.83$
	40.00	41.03	6.04		$S_R = 0.051\bar{X} + 3.24$	42.18	3.71		$S_R = 0.056\bar{X} + 2.10$	41.46	3.30		$S_R = 0.037\bar{X} + 2.97$
	80.00	81.40	5.86	5.44	$S_r = 0.061\bar{X} - 0.64$	79.97	6.66	5.28	$S_r = 0.040\bar{X} + 2.15$	81.63	6.94	5.65	$S_r = 0.058\bar{X} + 1.02$
	96.10	98.34	8.57			94.94	7.90			98.92	4.39		
	160.00	163.58	15.69	9.86		163.48	9.17	10.06		167.54	8.69	12.98	
	200.00	214.30	10.57			212.19	16.49			209.21	14.65		
Silver	0.80	0.93	0.09	0.14	$\bar{X} = 0.917C + 0.26$	0.70	0.34	0.34	$\bar{X} = 0.888C + 0.09$	0.70	0.26	0.10	$\bar{X} = 0.858C - 0.00$
	1.20	1.51	0.23		$S_R = 0.196\bar{X} - 0.09$	1.37	0.33		$S_R = 0.186\bar{X} + 0.17$	0.98	0.28		$S_R = 0.169\bar{X} + 0.14$
	48.00	49.39	3.25	1.81	$S_r = 0.053\bar{X} + 0.08$	45.43	6.78	5.15	$S_r = 0.164\bar{X} + 0.18$	45.59	4.27	2.70	$S_r = 0.120\bar{X} - 0.01$
	64.00	63.54	2.75			60.35	2.22			59.71	6.58		
	160.00	136.42	48.31	12.19		119.06	55.28	36.34		121.43	42.55	28.19	
	200.00	153.74	57.34			172.15	31.92			160.69	27.15		
Thallium	2.80	2.89	0.23	0.22	$\bar{X} = 0.984\bar{X} + 0.08$	2.88	0.40	0.16	$\bar{X} = 1.010C + 0.01$	2.88	0.14	0.12	$\bar{X} = 1.023C - 0.06$
	4.00	3.92	0.15		$S_R = 0.035\bar{X} + 0.09$	3.96	0.21		$S_R = 0.040\bar{X} + 0.21$	3.88	0.37		$S_R = 0.056\bar{X} + 0.04$
	20.00	19.27	0.99	0.67	$S_r = 0.027\bar{X} + 0.13$	19.77	1.13	0.83	$S_r = 0.039\bar{X} + 0.02$	20.22	1.05	0.65	$S_r = 0.049\bar{X} - 0.06$
	28.00	28.08	0.83			27.61	1.24			28.65	1.50		
	80.00	81.29	3.65	2.86		85.32	4.08	4.05		83.97	6.10	6.05	
	100.00	96.69	2.86			100.07	4.33			101.09	4.15		
Thorium	0.80	0.93	0.16	0.09	$\bar{X} = 1.013C + 0.08$	0.78	0.13	0.07	$\bar{X} = 1.019C - 0.06$	0.87	0.17	0.07	$\bar{X} = 1.069C - 0.03$
	1.20	1.22	0.19		$S_R = 0.036\bar{X} + 0.13$	1.09	0.19		$S_R = 0.035\bar{X} + 0.12$	1.15	0.17		$S_R = 0.041\bar{X} + 0.13$
	20.00	20.88	0.90	0.71	$S_r = 0.025\bar{X} + 0.07$	21.66	0.94	0.54	$S_r = 0.024\bar{X} + 0.05$	21.78	0.90	0.94	$S_r = 0.027\bar{X} + 0.04$
	28.00	27.97	1.11			28.09	0.83			29.86	1.65		
	80.10	81.14	2.99	2.14		79.99	2.03	2.60		86.00	3.43	1.95	
	100.00	102.64	3.39			100.50	4.56			107.35	4.72		

TABLE 12: SUMMARY STATISTICS AND DESCRIPTIVE EQUATIONS FOR THE 20 ANALYTES TESTED IN THE COLLABORATIVE STUDY

		Reagent Water				Finished Drinking Water				Ground Water			
Analyte	C[a]	$\bar{X}$[b]	S_R	S_r	Regr. Equations	$\bar{X}$	S_R	S_r	Regr. Equations	$\bar{X}$	S_R	S_r	Regr. Equations
Uranium	0.80	0.86	0.05	0.08	$\bar{X} = 1.026\underline{C} - 0.02$	0.85	0.15	0.09	$\bar{X} = 1.026\underline{C} - 0.04$	0.84	0.23	0.19	$\bar{X} = 1.058\underline{C} - 0.06$
	1.20	1.10	0.11		$S_R = 0.048\bar{X} + 0.02$	1.05	0.13		$S_R = 0.044\bar{X} + 0.11$	1.10	0.14		$S_R = 0.039\bar{X} + 0.17$
	20.10	21.38	0.99	0.82	$S_r = 0.027\bar{X} + 0.05$	22.30	1.40	0.46	$S_r = 0.022\bar{X} + 0.07$	21.56	1.11	1.08	$S_r = 0.028\bar{X} + 0.16$
	28.10	28.36	1.10			28.89	1.47			29.86	1.83		
	80.30	82.47	4.03	2.16		80.31	2.00	2.71		85.01	3.76	2.00	
	100.00	103.49	5.24			100.70	5.30			106.47	3.74		
Vanadium	32.00	31.02	2.68	2.19	$\bar{X} = 1.025C - 2.21$	33.15	2.51	2.28	$\bar{X} = 1.022\underline{C} - 0.30$	33.25	3.83	1.87	$\bar{X} = 1.076\underline{C} - 1.87$
	40.00	38.54	2.94		$S_R = 3.79$[d]	40.20	1.88		$S_R = 0.023\bar{X} + 1.45$	40.34	3.08		$S_R = 0.033\bar{X} + 2.25$
	80.00	79.14	4.94	4.29	$S_r = 3.26$[d]	77.83	4.18	2.75	$S_r = 0.023\bar{X} + 1.38$	84.42	3.97	2.93	$S_r = 0.049\bar{X} - 0.09$
	96.00	93.47	3.85			96.32	1.34			98.70	5.03		
	160.00	162.43	5.67	3.30		161.89	7.63	6.56		170.94	9.09	11.55	
	200.00	208.20	2.65			214.91	5.89			217.90	11.36		
Zinc	8.00	8.33	2.56	1.78	$\bar{X} = 1.042\underline{C} + 0.87$	11.60	6.18	5.72	$\bar{X} = 0.943\underline{C} + 2.54$	7.29	1.12	2.20	$\bar{X} = 0.962\underline{C} + 0.07$
	12.00	15.49	4.18		$S_R = 0.041\bar{X} + 2.60$	10.21	4.96		$S_R = 0.048\bar{X} + 5.27$	12.66	3.24		$S_R = 0.093\bar{X} + 0.92$
	56.00	56.07	2.91	2.47	$S_r = 0.030\bar{X} + 1.42$	56.83	7.66	4.56	$S_r = 0.004\bar{X} + 5.66$[c]	54.86	5.12	7.24	$S_r = 0.069\bar{X} + 1.55$
	80.00	85.53	5.81			82.88	8.34			78.62	8.56		
	160.00	165.17	7.78	9.87		156.69	17.01	9.48		150.12	12.52	10.84	
	200.00	207.27	14.61			191.59	17.21			184.37	16.59		

[a] True Value for the concentration added ($\mu g/L$)
[b] Mean Recovery ($\mu g/L$)
[c] $COD_w < 0.5$ - Use of regression equation outside study concentration range not recommended.
[d] $COD_w < 0$ - Mean precision is reported.
[e] $COD_w < 0$ - Unweighted linear regression equation presented.

TABLE 13: BACKGROUND AND SPIKE MEASUREMENTS IN WASTEWATER DIGESTATE[a]

	Background		Concentrate 1					Concentrate 2					
	Conc. µg/L	Std Dev µg/L	Spike µg/L	Found µg/L	Std Dev µg/L	% Rec %	RSD %	Spike µg/L	Found µg/L	Std Dev µg/L	% Rec %	RSD %	RSD_r %
Be	0.0	0.0	100	94.5	11.8	94.5	12.5	125	118.1	14.7	94.5	12.4	3.5
Al	78.2	12.4	200	260.9	41.2	91.4	15.8	250	309.1	48.5	92.4	15.7	2.7
Cr	19.5	8.1	200	222.2	23.3	101.4	10.5	250	274.3	26.6	101.9	9.7	2.0
V	1.9	2.8	250	271.8	36.5	108.0	13.4	200	219.3	30.1	108.7	13.7	2.6
Mn	296.6	24.7	125	419.0	35.7	97.9	8.5	100	397.4	34.8	100.8	8.8	1.0
Co	2.5	0.4	125	124.7	12.3	97.8	9.9	101	100.7	9.4	97.2	9.3	2.8
Ni	47.3	5.0	125	161.7	4.9	91.5	3.0	100	142.7	5.6	95.4	3.9	2.1
cu	77.4	13.2	125	194.5	29.5	93.7	15.2	100	172.3	26.6	94.9	15.4	2.2
Zn	77.4	4.9	200	257.4	16.3	90.0	6.3	250	302.5	21.1	90.0	7.0	1.8
As	0.8	1.1	200	194.9	8.0	97.1	4.1	250	244.7	12.8	97.6	5.2	3.4
Se	4.5	6.2	250	236.8	14.2	92.9	6.0	200	194.3	9.3	94.9	4.8	3.8
Mo	166.1	9.4	100	269.8	19.0	103.7	7.0	125	302.0	18.0	108.7	6.0	1.5
Ag	0.6	0.7	200	176.0	14.6	87.7	8.3	250	214.6	17.8	85.6	8.3	2.3
Cd	2.7	1.1	125	117.0	4.8	91.4	4.1	100	96.6	3.2	93.9	3.3	2.9
Sb	3.3	0.2	100	100.2	4.8	96.9	4.8	125	125.9	4.3	98.1	3.4	1.8
Ba	68.6	3.3	250	321.0	19.4	101.0	6.0	200	279.3	17.2	105.4	6.2	2.5
Tl	0.1	0.1	100	103.3	8.0	103.2	7.7	125	129.2	8.9	103.3	6.9	2.1
Pb	6.9	0.5	125	135.1	7.8	102.6	5.8	100	110.3	6.3	103.4	5.7	1.8
Th	0.1	0.1	125	140.2	19.5	112.1	13.9	100	113.3	15.4	113.2	13.6	2.7
U	0.4	0.2	125	141.2	19.3	112.6	13.7	100	113.6	16.0	113.2	14.1	2.5

[a]Results from 10 participating laboratories. Wastewater digestate supplied with the study materials. Mean background concentrations determined by the participants.

TABLE 14: SPIKE MEASUREMENTS IN PARTICIPANTS WASTEWATER[a]

	Concentrate 1					Concentrate 2					
	Spike µg/L	Found µg/L	Std Dev µg/L	% Rec %	RSD %	Spike µg/L	Found µg/L	Std Dev µg/L	% Rec %	RSD %	RSD_r %
Be	101	103.4	12.0	103.4	11.6	125	128.2	13.6	102.6	10.6	2.4
Al	200	198.7	23.9	99.4	12.0	250	252.4	15.5	101.0	6.1	2.9
Cr	200	205.4	12.3	102.7	6.0	250	253.4	15.4	101.4	6.1	1.1
V	250	246.5	4.4	98.6	1.8	200	196.8	2.8	98.4	1.4	2.0
Mn	125	119.0	5.4	95.2	4.5	100	95.5	4.3	95.5	4.5	0.8
Co	125	125.8	7.0	100.6	5.6	101	99.5	5.3	98.5	5.3	1.8
Ni	125	127.4	9.7	101.9	7.6	100	101.0	7.5	101.0	7.4	1.7
cu	125	126.8	5.3	101.4	4.2	100	105.3	3.6	105.3	3.4	2.8
Zn	200	201.4	36.7	100.7	18.2	250	246.4	29.7	98.6	12.1	2.6
As	200	207.3	11.9	103.7	5.7	250	263.0	2.6	105.2	1.0	3.2
Se	250	256.8	26.4	102.7	10.3	200	214.0	18.7	107.3	8.7	3.6
Mo	100	98.6	4.6	98.6	4.7	125	123.2	6.7	98.6	5.4	2.2
Ag	200	200.7	48.9	100.4	24.4	250	231.2	63.5	92.5	27.5	8.2
Cd	125	123.2	11.5	98.6	9.3	100	95.8	2.9	95.8	3.0	5.8
Sb	100	92.2	4.4	92.2	4.8	125	119.0	1.0	95.2	0.8	2.8
Ba	250	245.2	12.8	98.1	5.2	200	204.7	12.1	102.4	5.9	2.1
Tl	100	100.0	0.9	100.0	0.9	125	128.0	6.0	102.4	4.7	3.5
Pb	125	125.8	5.1	100.6	4.1	100	100.8	2.7	100.8	2.7	2.2
Th	125	124.2	7.6	99.4	6.1	100	99.8	5.7	99.8	5.7	3.2
U	125	130.4	10.3	104.3	7.9	100	106.4	6.8	106.4	6.4	2.3

[a]Results from five participating laboratories. Mean concentrations before spiking are not listed because they varied considerably among the different wastewaters.

Food

BAM: Yeasts, Molds and Mycotoxins

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January 2001

Bacteriological Analytical Manual
Chapter 18
Yeasts, Molds and Mycotoxins

Authors

The large and diverse group of microscopic foodborne yeasts and molds (fungi) includes several hundred species. The ability of these organisms to attack many foods is due in large part to their relatively versatile environmental requirements. Although the majority of yeasts and molds are obligate aerobes (require free oxygen for growth), their acid/alkaline requirement for growth is quite broad, ranging from pH 2 to above pH 9. Their temperature range (10-35°C) is also broad, with a few species capable of growth below or above this range. Moisture requirements of foodborne molds are relatively low; most species can grow at a water activity (a_w) of 0.85 or less, although yeasts generally require a higher water activity.

Both yeasts and molds cause various degrees of deterioration and decomposition of foods. They can invade and grow on virtually any type of food at any time; they invade crops such as grains, nuts, beans, and fruits in fields before harvesting and during storage. They also grow on processed foods and food mixtures. Their detectability in or on foods depends on food type, organisms involved, and degree of invasion; the contaminated food may be slightly blemished, severely blemished, or completely decomposed, with the actual growth manifested by rot spots of various sizes and colors, unsightly scabs, slime, white cottony mycelium, or highly colored sporulating mold. Abnormal flavors and odors may also be produced. Occasionally, a food appears mold-free but is found upon mycological examination to be contaminated. Contamination of foods by yeasts and molds can result in substantial economic losses to producer, processor, and consumer.

Several foodborne molds, and possibly yeasts, may also be hazardous to human or animal health because of their ability to produce toxic metabolites known as mycotoxins. Most mycotoxins are stable compounds that are not destroyed during food processing or home cooking. Even though the generating organisms may not survive food preparation, the preformed toxin may still be present. Certain foodborne molds and yeasts may also elicit allergic reactions or may cause infections. Although most foodborne fungi are not infectious, some species can cause infection, especially in immunocompromised populations, such as the aged and debilitated, HIV-infected individuals, and persons receiving chemotherapy or antibiotic treatment.

The dilution plating and the direct plating methods may be used to detect fungi in foods. The direct plating method is more efficient than the dilution plating method for detecting individual mold species, including most of the toxin producers, but it is less effective in detecting yeasts. It is also used to determine whether the presence of mold is due to external contamination or internal invasion. Methodology for testing the ability of isolates of toxigenic mold species to produce mycotoxins on sterile rice water substrate is included here.

Enumeration of Yeasts and Molds in Food--Dilution Plating Technique

A. **Equipment and materials**

1. Basic equipment (and appropriate techniques) for preparation of sample homogenate, see Chapter 1 [2]
2. Equipment for plating samples, see Chapter 3 [3]
3. Incubator, 25°C
4. Arnold steam chest
5. pH meter
6. Water bath, 45 ± 1° C

B. **Media [4] and reagents [5]**
Media

1. Dichloran rose bengal chloramphenicol (DRBC) agar (M183)
2. Dichloran 18% glycerol (DG18) agar (M184)
3. Plate count agar (PCA), standard methods (M124); add 100 mg chloramphenicol/liter when this medium is used for yeast and mold enumeration. This medium is not efficient when "spreader" molds are present.
4. Malt agar (MA)(M185)
5. Malt extract agar (Yeasts and Molds) (MEAYM)(M182)
6. Potato dextrose agar (PDA), dehydrated; commercially available (M127)

Antibiotic solutions

Antibiotics are added to mycological media to inhibit bacterial growth. Chloramphenicol is the antibiotic of choice, because it is stable under autoclave conditions. Therefore, media preparation is easier and faster due to the elimination of the filtration step. The recommended concentration of this antibiotic is 100 mg/liter medium. If bacterial overgrowth is apparent, prepare media by adding 50 mg/liter chloramphenicol before autoclaving and 50 mg/liter filter-sterilized chlortetracycline when the media have been tempered, right before pouring plates.

Prepare stock solution by dissolving 0.1 g chloramphenicol in 40 ml distilled water; add this solution to 960 ml medium mixture before autoclaving. When both chloramphenicol and chlortetracycline are used, add 20 ml of the above chloramphenicol stock solution to 970 ml medium before autoclaving. Then, prepare chlortetracycline stock solution by dissolving 0.5 g antibiotic in 100 ml distilled water and filter sterilize. Use 10 ml of this solution for each 990 ml of autoclaved and tempered medium. Refrigerate in the dark and re-use remaining stock solutions for up to a month. Stock solutions should be brought to room temperature before adding to tempered medium.

Procedures

Sample preparation

Analyze 25-50 g from each subsample; generally, larger sample sizes increase reproducibility and lower variance compared with small samples. Test individual subsamples or composite according to respective Compliance Program for the food under analysis. Add appropriate amount of 0.1% peptone water to the weighed sample to achieve 10^{-1} dilution, then homogenize in a stomacher for 2 min. Alternatively, blending for 30-60 sec can be used but is less effective. Make appropriate 1:10 (1+9) dilutions in 0.1% peptone water. Dilutions of 10^{-6} should suffice.

Plating and incubation of sample

Spread-plate method. Aseptically pipet 0.1 ml of each dilution on pre- poured, solidified DRBC agar plates and spread inoculum with a sterile, bent glass rod. DG18 is preferred when the water activity of the analyzed sample is less than 0.95. Plate each dilution in triplicate.

Pour-plate method. Use sterile cotton-plugged pipet to place 1.0 ml portions of sample dilution into prelabeled 15 x 100 mm Petri plates (plastic or glass), and immediately add 20-25 ml tempered DG18 agar. Mix contents by gently swirling plates clockwise, then counterclockwise, taking care to avoid spillage on dish lid. After adding sample dilution, add agar within 1-2 min; otherwise, dilution may begin to adhere to dish bottom (especially if sample is high in starch content and dishes are plastic) and may not mix uniformly. Plate each dilution in triplicate.

From preparation of first sample dilution to pouring or surface-plating of final plate, no more than 20 min (preferably 10 min) should elapse. **Note:** Spread plating of diluted sample is considered better than the pour plate method. When the pour plate technique is used, fungal colonies on the surface grow faster and often obscure those underneath the surface, resulting in less accurate enumeration. Surface plating gives a more uniform growth and makes colony isolation easier. DRBC agar should be used for spread plates only.

Incubate plates in the dark at 25°C. Do not stack plates higher than 3 and do not invert. **Note:** Let plates remain undisturbed until counting.

Counting of plates

Count plates after 5 days of incubation. If there is no growth at 5 days, re-incubate for another 48 h. Do not count colonies before the end of the incubation period because handling of plates could result in secondary growth from dislodged spores, making final counts invalid. Count plates containing 10-150 colonies. If mainly yeasts are present, plates with 150 colonies are usually countable. However, if substantial amounts of mold are present, depending on the type of mold, the upper countable limit may have to be lowered at the discretion of the analyst. Report results in colony forming units (CFU)/g or CFU/ml based on average count of triplicate set. Round off counts to two significant figures. If third digit is 6 or above, round off to digit above (e.g., 456 = 460); if 4 or below, round off to digit below (e.g., 454 = 450). If third digit is 5, round off to digit below if first 2 digits are an even number (e.g., 445 = 440); round off to digit above if first 2 digits are an odd number (e.g., 455 = 460). When plates from all dilutions have no colonies, report mold and yeast counts (MYC) as less than 1 times the lowest dilution used.

Isolate individual colonies on PDA or MA, if further analysis and species identification is necessary.

Enumeration of Molds in Foods--Direct Plating Technique for Foods That Can Be Handled with Forceps (Dried Beans, Nuts, Whole Spices, Coffee and Cocoa Beans, etc.)

A. **Equipment and materials**

1. Freezer, -20° C
2. Beakers, sterile, 300 ml
3. Forceps, sterile
4. Arnold steam chest
5. Water bath, 45 ± 1° C
6. Incubator, 25° C

B. **Media and reagents**

1. Dichloran rose bengal chloramphenicol (DRBC) agar (M183)
2. Dichloran 18% glycerol (DG18) agar (M184)
3. Antibiotic solutions (see previous section)
4. NaOCl (commercial bleach) solution, 10%
5. Sterile distilled water

C. **Analysis of non-surface-disinfected (NSD) foods**

Sample and media preparation

Before plating, hold sample at -20° C for 72 h to kill mites and insects that might interfere with analysis.

Prepare DRBC agar as described in the appendix. If DRBC is not available, or the water activity of the analyzed sample is less than 0.95, use DG18 agar. Media should be prepared no more than 24 h prior to use.

Plating and incubation of sample

From each sample, transfer about 50 g into a sterile 300 ml beaker. Using 95% ethanol-flamed forceps place intact food items on surface of solidified agar, 5-10 items per plate (depending on size of food item) 50 items total per sample.

Flame forceps between plating of each item. Use several forceps alternately to avoid overheating. Do not plate visibly moldy or otherwise blemished items.

Align 3-5 plates in stacks and identify with sample number plus date of plating. Incubate stacks, undisturbed in the dark at 25°C for 5 days. If there is no growth at 5 days of incubation, re-incubate for another 48 h to allow heat- or chemically-stressed cells and spores enough time to grow.

Reading of plates

Determine occurrence of mold in percentages. If mold emerged from all 50 food items, moldiness is 100%; if from 32 items, moldiness is 64%. Determine percent occurrence of individual mold genera and species in like manner. Experienced analysts may identify *Aspergillus,*

Penicillium and most other foodborne mold genera directly on medium with low power (10-30X) magnification.

D. **Analysis of surface-disinfected (SD) foods**
Perform disinfection in clean laboratory sink, not stainless steel, free from any acid residues, with tap water running (precautions against chlorine gas generation). Wear rubber gloves and transfer about 50 g of sample into a sterile 300 ml beaker. Cover with 10% chlorine (commercial bleach) solution for 2 min, while swirling beaker contents gently but constantly in clockwise-counterclockwise motion. Decant 10% chlorine solution and give beaker contents two 1-min rinses with sterile distilled water. Prepare plates; plate sample, incubate, and read plates as in non-surface disinfected direct plating method, above. Compare NSD and SD results from the same sample to determine if moldiness was due mainly to surface contamination or to internal invasion and growth. Isolate individual colonies on PDA or MA.

Fluorescence Microscopy Procedure for Quantitation of Viable and Nonviable Yeasts in Beverages

Methods for counting viable yeasts by plating are described above. A direct microscopic procedure for counting nonviable and viable yeasts in beverages and other liquid samples is presented here. Quantitating yeast cells by microscopy eliminates the need for extended incubation, thus reducing the analytical time required. All yeasts can be counted, and living and dead yeast cells can be differentiated.

A. **Equipment and materials**

1. Millipore disk filter holders for standard syringes
2. Millipore filters: AABG, 0.8 µm, black, gridded; 25 mm diameter
3. Syringes, disposable
4. Pipets
5. Forceps
6. Bibulous paper
7. Microscope slides and 24 x 24 mm coverslips
8. Fluorescence microscope: blue excitation; l0X eyepieces with Howard mold count or other eyepiece grid; 20X or 40X objective

B. **Reagents**

1. Aniline blue; 1% in M/15 K_2HPO_4 (M/15 is equivalent to 11.6 g/L), adjusted to pH 8.9 with K_3PO_4. A stock solution can be made; age improves fluorescence.
2. NaOH; 25 g in 100 ml water

C. **Sample preparation for filterable liquids (e.g. water and grape juice)**
Filter aliquot (usually 10 ml) of sample through Millipore filter (AABG, 0.8 µm, black, gridded).(Portion size can be increased or decreased, depending on level of contamination). Use Millipore disk filter holder which attaches to standard syringe. Make sure that syringe is accurate. If not, remove plunger, attach syringe to filter holder, and pipette 10 ml into syringe. Press all of sample through filter. Do this with air cushion of about 3 ml between plunger and sample. Keep filter holder vertical to ensure even distribution of sample over filter. Remove filter from filter holder and place on microscope slide; grid should be parallel to edges of slide to facilitate counting.

D. **Sample preparation for non-filterable liquids that clog the filter (e.g. orange juice)**
To suppress background interference in fluorescence microscope, mix 4 ml sample with 1 ml sodium hydroxide (25 g in 100 ml water). Shake well and wait 10 min. Place Millipore filter (AABG, 0.8 µm, black, gridded) on a piece of bibulous paper and spread 0.1 or 0.01 ml (depending on level of contamination) of sample over filter. When filter surface is dry, place filter on microscope slide, keeping grid parallel to edges of slide to facilitate counting.

E. **Microscopic counting procedure**

Cover filter with a drop of aniline blue, 1% in M/15 (11.6 g/L) K_2HPO_4, adjusted to pH 8.9 with K_3PO_4. Spread aniline blue stain over whole filter with glass rod or coverslip without touching filter itself. Wait about 5 min; then cover filter with 24 x 24 mm coverslip.

Count yeasts, using fluorescence microscope with blue excitation. Use 10X eyepiece with Howard mold count or other eyepiece grid, and 20X (or 40X) objective. Count 3 squares of eyepiece grid in each field of filter not covered by gasket. Count budding yeasts as 1 cell if daughter cell is obviously smaller than mother cell. If they are approximately equal in size, count them as 2 cells. Count all yeasts located completely within an eyepiece square and all yeasts touching left and lower border of eyepiece square. Do not count yeasts touching right and upper borders.

This method also differentiates dead (heat- or formaldehyde-killed) and living yeast cells. Dead cells show fairly uniform fluorescence, and plasma may be granular. In living cells, the cell wall stains brighter and is more defined than the plasma, which is less prominent and uniformly stained.

F. **Calculations to determine number of yeasts per ml**

Determine area of filter covered by 1 square of eyepiece grid, using objective (stage) micrometer. For filtered samples, the working area of the Millipore filter (portion not covered by the gasket) is 380 mm^2. For nonfiltered samples, it is the entire filter, or 491 mm^2, since no gasket is used.

$$\text{No. of yeasts per ml} = \frac{\text{No. of yeasts counted}}{\text{No. of eyepiece squares examined}} \times \frac{\text{working area of filter}}{\text{area of one eyepiece square}} \times \frac{1}{\text{volume of liquid}}$$

G. **NOTE:** For non-filterable liquids, volume includes only net amount used and not volume of NaOH added (i.e., 80% of total volume applied to filter).

H. For background information on the method, including photographs of dead and living yeast cells, **see** Koch et al., ref. 8, below.

Methods for Determining Toxin Production by Molds

A. **Equipment and materials**

1. Erlenmeyer flasks, 300 ml, wide-mouth
2. Cotton, nonabsorbent
3. Funnels, short-stem glass, 90-100 mm diameter
4. Filter paper, 18 cm diameter, folded (Schleicher & Schuell No. 588)
5. Boiling chips, silicon carbide
6. Fume hood equipped with steam bath; air-flow rate, 100 cubic ft/min
7. Blender, high speed, explosion-proof
8. Thin layer chromatographic apparatus or high-performance liquid chromatograph
9. Incubator, 22-25°C

B. **Media and reagents**

1. Long or short grain polished rice
2. Chloroform for extraction of aflatoxins, ochratoxins, sterigmatocystin, xanthomegnin, luteoskyrin, patulin, penicillic acid, citrinin, T-2 toxin, zearalenone
3. Methanol for extraction of deoxynivalenol
4. Appropriate mycotoxin standards
5. NaOCl solution, 5%

C. **Toxin production**

Into 300 ml wide-mouth Erlenmeyer flask, add 50 g rice and 50 ml distilled water. Plug flasks with cotton and autoclave 20 min at 121°C and 15 psi. Aseptically multispore-inoculate separate cooled flasks with individual mold isolates. Incubate inoculated flasks at 22-25°C until entire surface is covered with growth, and mycelium has penetrated to bottom of flask (15-20 days). To each flask, add 150 ml chloroform (150 ml methanol if toxin in question is deoxynivalenol), using short-stem glass funnel inserted alongside unremoved cotton plug (to minimize mold spore dissemination). Heat flask contents in fume hood on steam bath until solvent begins to boil. (Conduct all subsequent steps in fume hood.) With spatula, break up moldy rice cake and transfer flask contents into explosion-proof blender and blend at high speed for 1 min. Filter blender contents through filter paper inserted into short-stem glass funnel. Collect filtrate in 300 ml Erlenmeyer flask. Return rice cakes to blender, add 100 ml unheated solvent and blend 1 min at high speed. Filter as above and combine filtrates. Add boiling chips to flask containing filtrates and evaporate with steam to 20-25 ml. If analysis is not to follow immediately, evaporate to dryness and store flask in the dark. Rinse all glassware, etc., used for extraction in 5% NaOCl solution before soap and water cleansing. Submerge rice cake in 5% NaOCl solution for 72 h before autoclaving and disposal.

D. **Toxin analysis**

Appropriate mycotoxin standards are required for both qualitative and quantitative analysis of toxin. Use either thin layer chromatography as described in references 16 or 17 or high performance liquid chromatography,as described in reference 15a, to determine mycotoxins extracted from mold cultures. Naturally occurring mycotoxins in foods or feeds can best be determined by methods described in *Official Methods of Analysis* (16).

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Media

Dichloran 18% glycerol (DG18) agar(M184)

Glucose	10.0 g
Peptone	5.0 g

KH_2PO_4	1.0 g
$MgSO_4.7H_2O$	0.5 g
Dichloran (2,6-dichloro-4-nitroaniline) solution (0.2% (w/v) in ethanol)	1.0 ml
Chloramphenicol	0.1 g
Agar	15.0 g
Distilled water	800 ml

Mix above items and steam to dissolve agar, then bring volume to 1000 ml with distilled water. Add 220 g glycerol (analytical reagent grade), and sterilize by autoclaving at 121°C for 15 min. Temper medium to 45° C and pour plates under aseptic conditions. The final a_w of this medium is 0.955. DG18 agar is used as a general purpose mold enumeration medium and is preferred when the a_w of the analyzed food is less than 0.95. The low water activity of this medium reduces interference by bacteria and fast-growing fungi. When both yeasts and molds must be enumerated, DRBC agar should be used.

Dichloran rose bengal chloramphenicol (DRBC) agar (M183)

Glucose	10.0 g
Bacteriological peptone	5.0 g
KH_2PO_4	1.0 g
$MgSO_4.7H_2O$	0.5 g
Rose bengal (5% aqueous soln., w/v)	0.5 ml
Dichloran (0.2% in ethanol, w/v)	1.0 ml
Chloramphenicol	0.1 g
Agar	15.0 g
Distilled water	1.0 liter

Final pH should be 5.6

Mix ingredients, heat to dissolve agar and sterilize by autoclaving at 121°C for 15 min. Temper to 45 ± 1° C in a water bath and pour plates.

Notes: DRBC agar is especially useful for analyzing samples containing "spreader" molds (e.g. *Mucor, Rhizopus*, etc.), since the added dichloran and rose bengal effectively slow down the growth of fast-growing fungi, thus readily allowing detection of other yeast and mold propagules, which have lower growth rates.

Media containing rose bengal are **light-sensitive**; relatively short exposure to light will result in the formation of inhibitory compounds. Keep these media in a dark, cool place until used. DRBC agar should be used for spread plates only.

Malt Agar (MA)(M185)

Malt extract, powdered	20.0 g
Agar	20.0 g
Distilled water	1.0 liter

Mix ingredients, steam to dissolve agar and sterilize for 15 min at 121° C. Temper medium to 45° C and pour plates under aseptic conditions. To prepare slants dispense 5-6 ml of steamed medium (before autoclaving) into each of several 16 x 125 mm screw-cap tubes, loosely cap tubes and sterilize as above. After autoclaving lay tubes in a slanting position and let them cool. This medium is recommended as a general maintenance medium.

Malt Extract Agar (Yeasts and Molds)(MEA)	**(M182)**
Malt extract, powdered	20.0 g
Glucose	20.0 g
Peptone	1.0 g
Agar	20.0 g
Distilled water	1.0 liter

Mix ingredients, heat to dissolve agar and sterilize at 121° C for 15 min. Temper media to 45° C and pour plates under aseptic conditions. Dehydrated MEA is commercially available, but since more than one MEA formula exists, check for the appropriate composition. This medium is recommended for the identification of *Aspergillus* and *Penicillium*.

Hypertext Source: Bacteriological Analytical Manual, 8th Edition, Revision A, 1998. Chapter 18 .
*Authors: Valerie Tournas, Michael E. Stack, Philip B. Mislivec, Herbert A. Koch and Ruth Bandler
Revised: 2000-APR-17

Links on this page:

1. http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm
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APPENDIX C
Intake Report

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ESTIMATED DAILY INTAKE OF CRANBERRY EXTRACT POWDER BY THE U.S. POPULATION FROM PROPOSED FOOD-USES (2013-2014 NHANES)

CONFIDENTIAL

Draft for Discussion

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Estimated Daily Intake of Cranberry Extract Powder by the U.S. Population from Proposed Food-Uses (2013-2014 NHANES)

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Estimated Daily Intake of Cranberry Extract Powder by the U.S. Population from Proposed Food-Uses (2013-2014 NHANES)

1.0 INTRODUCTION

Cranberry extract powder is proposed for use in the United States (U.S.) in beverages and beverage bases and processed fruits and fruit juices. The main components in cranberry extract powder include polyphenols, specifically proanthocyanidins (~55%), anthocyanins (5 to 8%), phenolics (35 to 38%), and organic acids (0.4 to 0.5%).

Estimates for the intake of cranberry extract powder and its polyphenols (as mentioned above) were based on the proposed food-uses and use-levels for cranberry extract powder in conjunction with food consumption data included in the U.S. National Center for Health Statistics (NCHS)'s National Health and Nutrition Examination Surveys (NHANES) 2013-2014. Calculations for the mean and 90th percentile *per capita* and consumer-only intakes were performed for all proposed food-uses of cranberry extract powder and the percentage of consumers were determined. Similar calculations were used to estimate the intake of cranberry extract powder resulting from each individual proposed food-use, including the calculations of percent consumers. In both cases, the per person and per kilogram body weight intakes were reported for the following population groups:

- Infants and young children, up to and including 2 years;
- Children, ages 3 to 11;
- Female teenagers, ages 12 to 19;
- Male teenagers, ages 12 to 19;
- Female adults, ages 20 and up;
- Male adults, ages 20 and up; and
- Total population (all age and gender groups combined).

2.0 FOOD CONSUMPTION SURVEY DATA

2.1 Survey Description

NHANES for the years 2013-2014 are available for public use (CDC, 2015, 2016; USDA, 2016). NHANES are conducted as continuous, annual surveys, and are released in 2-year cycles. During each year of the ongoing NHANES program, individuals from the U.S. are sampled from up to 30 different study locations in a complex multi-stage probability design intended to ensure the data are a nationally representative sample of the U.S. population.

NHANES 2013-2014 dietary survey data were collected from individuals and households *via* 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2) throughout all 4 seasons of the year. Day 1 data were collected in-person, and Day 2 data were collected by telephone in the following 3 to 10 days, on different days of the week, to achieve the desired degree of statistical independence. The data were collected by first selecting primary sampling units (PSUs), which were counties throughout the U.S., of which 30 PSUs are visited per year. Smaller contiguous counties were combined to attain a minimum population size. These PSUs were segmented, and households were chosen within each segment. One or more participants within a household were interviewed. For NHANES 2013-2014, 14,332 individuals were selected for the sample, 10,175 were interviewed (71.0%), and 9,813 were examined (68.5%).

In addition to collecting information on the types and quantities of foods being consumed, NHANES 2013-2014 collected socio-economic, physiological, and demographic information from individual participants in the survey, such as sex, age, body weight, and other variables (such as height and race-ethnicity) that may be useful in characterizing consumption. The inclusion of this information allows for further assessment of food intake based on consumption by specific population groups of interest within the total population. The primary sample design for NHANES 2013-2014 includes an oversample of non-Hispanic Asian persons, Hispanic persons, non-Hispanic black persons, older adults, and "low income whites/others"; however, sample weights were incorporated to allow estimates from these subgroups to be combined to obtain national estimates that reflect the relative proportions of these groups in the population as a whole (CDC, 2015, 2016; USDA, 2016).

2.2 Statistical Methods

For the intake assessment, consumption data from individual dietary records, detailing food items ingested by each survey participant, were collated by computer and used to generate estimates for the intake of cranberry extract powder by the U.S. population[1]. Estimates for the daily intake of cranberry extract powder represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES 2013-2014; these average amounts comprised the distribution from which mean and percentile intake estimates were determined. Mean and percentile estimates were generated incorporating survey weights in order to provide representative intakes for the entire U.S. population. "*Per capita*" intake refers to the estimated intake of cranberry extract powder averaged over all individuals surveyed, regardless of whether they consumed food products in which cranberry extract powder is proposed for use, and therefore includes individuals with "zero" intakes (*i.e.*, those who reported no intake of food products containing cranberry extract powder during the 2 survey days). "Consumer-only" intake refers to the estimated intake of cranberry extract powder by those individuals who reported consuming food products in which the use of cranberry extract powder cranberry extract powder is currently under consideration. Individuals were considered "consumers" if they reported consumption of 1 or more food products in which cranberry extract powder is proposed for use on either Day 1 or Day 2 of the survey.

[1] Statistical analysis and data management were conducted in DaDiet Software (Dazult Ltd., 2018). DaDiet Software is a web-based software tool that allows accurate estimate of exposure to nutrients and to substances added to foods, including contaminants, food additives and novel ingredients. The main input components are concentration (use-level) data and food consumption data. Data sets are combined in the software to provide accurate and efficient exposure assessments.

Mean and 90th percentile intake estimates based on sample sizes of less than 30 and 80, respectively, may not be considered statistically reliable due to the limited sampling size (CDC, 2013). As such, the reliability of estimates for the intake of cranberry extract powder based on consumption estimates derived from individual population groups of a limited sample size should be interpreted with caution. These values are marked with an asterisk in the relevant data tables.

3.0 FOOD USAGE DATA

The proposed food-uses and use-levels for cranberry extract powder employed in the current intake analysis are summarized in Table 3-1. Food codes representative of each proposed food-use were chosen from the NHANES 2013-2014 (CDC, 2016). Food codes were grouped in food-use categories according to Title 21, Section §170.3 of the Code of Federal Regulations (CFR, 2018a). All food codes included in the current intake assessment are listed in Appendix C.

The use-level of cranberry extract powder for beverages and beverage bases, and for coffee and tea are 150 mg/8 ounce serving or 62.5 mg/100 g. This is equivalent to:

- 33.3 mg/100 g of proanthocyanidins;
- 5.0 mg/100 g of anthocyanins;
- 25.4 mg/100 g of phenolics; and
- 0.4 mg/100 g of organic acids.

The use-level of cranberry extract powder for processed fruits and fruit juices is 300 mg/8 ounce serving or 125 mg/100 g. This is equivalent to:

- 66.6 mg/100 g of proanthocyanidins;
- 10.0 mg/100 g of anthocyanins;
- 50.8 mg/100 g of phenolics; and
- 0.8 mg/100 g of organic acids.

Table 3-1 Summary of the Individual Proposed Food-Uses and Use-Levels for Cranberry Extract Powder in the U.S.

Food Category (21 CFR §170.3)	Food-Uses	RACC[a] (mL)	Cranberry Extract Powder Level (mg/8 oz serving)	Cranberry Extract Powder Use-Levels (mg/100 g)
Beverages and Beverage Bases	Energy Drinks	360	150	62.5
	Enhanced or Fortified Waters	360	150	62.5
	Flavored or Carbonated Waters	360	150	62.5
	Sport and Electrolyte Drinks, Fluid Replacement Drinks	360	150	62.5
Coffee and Tea	Ready-to-Drink Cold brew Coffee Drinks	360	150	62.5
Processed Fruits and Fruit Juices	Fruit Drinks and Ades (Ready-to-Drink and Powder Mixes)	240	300	125

CFR = Code of Federal Regulations; RACC = Reference Amounts Customarily Consumed per Eating Occasion; U.S. = United States.
[a] RACC based on values established in 21 CFR §101.12 (CFR, 2018b). RACCs are included for reference; however, the assessment was conducted based on use-levels expressed per 100 g.

4.0 FOOD SURVEY RESULTS

4.1 Estimated Daily Intake from All Proposed Food Uses

Estimates for the total daily intakes of cranberry extract powder from proposed food-uses are provided in Tables 4.1-1 (mg/person/day) and 4.1-2 (mg/kg body weight/day). Estimates for the daily intake of cranberry extract powder from individual proposed food-uses in the U.S. are summarized in Tables A-1 to A-7 and B-1 to B-7 of Appendices A and B, respectively. Tables A-1 to A-7 provide estimates for the daily intake of cranberry extract powder on an absolute basis (mg/person/day), whereas Tables B-1 to B-7 provide estimates for the daily intake of cranberry extract powder on a per kilogram body weight basis (mg/kg body weight/day).

Estimates for consumers-only intake of the polyphenols and other components from cranberry extract powder from the proposed food-uses are provided in Tables 4.2-1 (mg/person/day) and 4.2-2 (mg/kg body weight/day).

4.1.1 Cranberry Extract Powder

Table 4.1.1-1 summarizes the estimated total intake of cranberry extract powder (mg/person/day) from all proposed food-uses in the U.S. population group. Table 4.1.1-2 presents this data on a per kilogram body weight basis (mg/kg body weight/day). The percentage of users was low among all age groups evaluated in the current intake assessment; ranging from 22.6 to 55.6% of the population groups consisted of users of those beverage products in which cranberry extract powder is currently proposed for use (Table 4.1.1-1). Children had the greatest proportion of consumers at 55.6%. The consumer-only intakes are more applicable to the assessment of safety as they are more likely to represent exposure in the target populations. Consequently, only the consumer-only intake results will be discussed in detail.

Among the total population (all ages), the mean and 90th percentile consumer-only intakes of cranberry extract powder were determined to be 332 and 639 mg/person/day, respectively. Of the individual population groups, male teenagers were determined to have the greatest mean at 449 mg/person/day, while male adults were determined to have the greatest 90th percentile consumer-only intakes of cranberry extract powder on an absolute basis at 856 mg/person/day, respectively. Infants and young children were identified to have the lowest mean and 90th percentile consumer-only intakes of 242 and 486 mg/person/day, respectively (Table 4.1.1-1).

Table 4.1.1-1 Summary of the Estimated Daily Intake of Cranberry Extract Powder from Proposed Food-Uses in the U.S. by Population Group (2013-2014 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (mg/day)		Consumer-Only Intake (mg/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants and Young Children	0 to 2	55	194	22.6	142	242	486
Children	3 to 11	147	407	55.6	721	263	518
Female Teenagers	12 to 19	148	388	46.4	281	319	590
Male Teenagers	12 to 19	211	504	46.9	274	449	808

Table 4.1.1-1 Summary of the Estimated Daily Intake of Cranberry Extract Powder from Proposed Food-Uses in the U.S. by Population Group (2013-2014 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (mg/day)		Consumer-Only Intake (mg/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Female Adults	20 and up	100	317	31.5	719	316	634
Male Adults	20 and up	113	350	30.1	673	375	856
Total Population	All ages	116	354	35.1	2,810	332	639

n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

On a body weight basis, the total population (all ages) mean and 90th percentile consumer-only intakes of cranberry extract powder were determined to be 5.8 and 12.9 mg/kg body weight/day, respectively. Among the individual population groups, infants and young children were identified as having the highest mean and 90th percentile consumer-only intakes of any population group, of 20.0 and 35.2 mg/kg body weight/day, respectively. Female adults had the lowest mean and 90th percentile consumer-only intakes of 4.2 and 8.8 mg/kg body weight/day, respectively (Table 4.1.1-2).

Table 4.1.1-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Cranberry Extract Powder from Proposed Food-Uses in the U.S. by Population Group (2013-2014 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants and Young Children	0 to 2	4.5	15.6	22.7	142	20.0	35.2
Children	3 to 11	5.3	15.1	55.5	716	9.5	18.1
Female Teenagers	12 to 19	2.4	6.6	46.3	277	5.1	10.6
Male Teenagers	12 to 19	3.0	7.3	47.2	273	6.4	13.1
Female Adults	20 and up	1.3	4.3	31.6	716	4.2	8.8
Male Adults	20 and up	1.3	4.2	30.2	671	4.3	9.1
Total Population	All ages	2.0	5.9	35.1	2,795	5.8	12.9

bw = body weight; n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

4.1.2 Polyphenols

Table 4.1.2-1 summarizes the estimated daily intake of polyphenols (proanthocyanidins, anthocyanins, phenolics, and organic acids) from cranberry extract powder (mg/person/day) from all proposed food-uses by consumers-only in the U.S. population group. Table 4.1.2-2 presents this data on a per kilogram body weight basis (mg/kg body weight/day).

Among the total population (all ages), the mean and 90th percentile consumer-only intakes of proanthocyanidins from cranberry extract powder were determined to be 177 and 340 mg/person/day, respectively. Of the individual population groups, male teenagers were determined to have the greatest mean at 239 mg/person/day, while male adults were determined to have the greatest 90th percentile intakes of proanthocyanidins from cranberry extract powder on an absolute basis at 456 mg/person/day, respectively. Infants and young children had the lowest mean and 90th percentile consumer-only intakes of 129 and 259 mg/person/day, respectively (Table 4.1.2-1).

Among the total population (all ages), the mean and 90th percentile intakes of anthocyanins from cranberry extract powder were determined to be 27 and 51 mg/person/day, respectively. The equivalent intakes of phenolics were 135 and 260 mg/kg body weight/day, respectively; while organic acid intakes from this ingredient were 2.1 and 4.1 mg/kg body weight/day, respectively.

Of the individual population groups, male teenagers were determined to have the greatest consumer-only mean intakes for anthocyanins, phenolics, and organic acids on an absolute basis of 36, 182, and 2.9 mg/person/day, respectively. At the 90th percentile, male adults were determined to have the greatest 90th percentile consumer-only intakes of anthocyanins, phenolics, and organic acids from cranberry extract powder at 69, 348, and 5.5 mg/person/day, respectively.

Table 4.1.2-1 Summary of the Estimated Daily Intake of Polyphenols – Proanthocyanidins, Anthocyanins, Phenolics, and Organic Acids from Cranberry Extract Powder from Proposed Food-Uses by Consumers Only in the U.S. by Population Group (2013-2014 NHANES Data)

Population Group	Age Group (Years)	% (n)	Proanthocyanidins (mg/day)		Anthocyanins (mg/day)		Phenolics (mg/day)		Organic Acids (mg/day)	
			Mean	P90	Mean	P90	Mean	P90	Mean	P90
Infants and Young Children	0 to 2	22.6 (142)	129	259	19	39	98	197	1.6	3.1
Children	3 to 11	55.6 (721)	140	276	21	41	107	211	1.7	3.3
Female Teenagers	12 to 19	46.4 (281)	170	314	25	47	129	240	2.0	3.8
Male Teenagers	12 to 19	46.9 (274)	239	431	36	65	182	328	2.9	5.2
Female Adults	20 and up	31.5 (719)	168	338	25	51	128	258	2.0	4.1
Male Adults	20 and up	30.1 (673)	200	456	30	69	152	348	2.4	5.5
Total Population	All ages	35.1 (2,810)	177	340	27	51	135	260	2.1	4.1

n = sample size; NHANES = National Health and Nutrition Examination Survey; P90 = 90th percentile; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

On a body weight basis, the total population (all ages) mean and 90th percentile intakes of proanthocyanidins from cranberry extract powder were determined to be 3.1 and 6.9 mg/kg body weight/day, respectively (Table 4.1.2-2). Among the individual population groups, infants and young children were identified as having the highest mean and 90th percentile proanthocyanidin intakes of any population group, of 10.7 and 18.8 mg/kg body weight/day, respectively. Female adults had the lowest mean and 90th percentile consumer-only intakes of 2.2 and 4.7 mg/kg body weight/day, respectively.

The total population (all ages) mean and 90th percentile intakes of anthocyanins from cranberry extract powder were determined to be 0.5 and 1.0 mg/kg body weight/day, respectively. The equivalent intakes of phenolics were 2.4 and 5.3 mg/kg body weight/day, respectively; while organic acid intakes from this ingredient were 0.04 and 0.08 mg/kg body weight/day, respectively.

Among the individual population groups, infants and young children were identified as having the highest mean consumer-only intakes of anthocyanins, phenolics and organic acids at 1.6, 8.1, and 0.13 mg/kg body weight/day, respectively. This age group was also determined to have the highest 90th percentile intakes of anthocyanins, phenolics and organic acids at 2.8, 14.3, and 0.23 mg/kg body weight/day, respectively.

Table 4.1.2-2 Summary of the Estimated Daily Intake of Polyphenols – Proanthocyanidins, Anthocyanins, Phenolics, and Organic Acids from Cranberry Extract Powder from Proposed Food-Uses by Consumers Only in the U.S. by Population Group (2013-2014 NHANES Data)

Population Group	Age Group (Years)	% (n)	Proanthocyanidins (mg/kg bw/day)		Anthocyanins (mg/kg bw/day)		Phenolics (mg/kg bw/day)		Organic acids (mg/kg bw/day)	
			Mean	P90	Mean	P90	Mean	P90	Mean	P90
Infants and Young Children	0 to 2	22.7 (142)	10.7	18.8	1.6	2.8	8.1	14.3	0.13	0.23
Children	3 to 11	55.5 (716)	5.1	9.7	0.8	1.5	3.9	7.4	0.06	0.12
Female Teenagers	12 to 19	46.3 (277)	2.7	5.6	0.4	0.8	2.1	4.3	0.03	0.07
Male Teenagers	12 to 19	47.2 (273)	3.4	7.0	0.5	1.0	2.6	5.3	0.04	0.08
Female Adults	20 and up	31.6 (716)	2.2	4.7	0.3	0.7	1.7	3.6	0.03	0.06
Male Adults	20 and up	30.2 (671)	2.3	4.9	0.3	0.7	1.7	3.7	0.03	0.06
Total Population	All ages	35.1 (2,795)	3.1	6.9	0.5	1.0	2.4	5.3	0.04	0.08

n = sample size; NHANES = National Health and Nutrition Examination Survey; P90 = 90th percentile; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

4.2 Estimated Daily Intake of Cranberry Extract Powder from Individual Proposed Food-Uses in the U.S.

Estimates for the mean and 90th percentile daily intakes of cranberry extract powder from each individual food category are summarized in Tables A-1 to A-7 and B-1 to B-7 on a mg/day and mg/kg body weight/day basis, respectively. The total U.S. population was identified as being significant consumers of fruit drinks and ades (17.1 to 49.2% consumers) and sport or electrolyte drinks, fluid replacement drinks (3.3 to 19.8% consumers).

In terms of contribution to total mean intake of cranberry extract powder, fruit drinks and ades (which contributed 52.0 to 83.5% to total mean intakes) and sport or electrolyte drinks, fluid replacement drinks (which contributed 8.3 to 41.2% to total mean intakes) were the 2 main sources of intake across all population groups. Energy drinks, enhanced or fortified waters, flavored or carbonated waters, and ready-to-drink cold brew coffee drinks all individually contributed ≤13.6% to total mean cranberry extract powder intakes across all population groups (see Tables A-1 to A-7 and/or B-1 to B-7 for further details).

5.0 SUMMARY AND CONCLUSIONS

Consumption data and information pertaining to the individual proposed food-uses of cranberry extract powder were used to estimate the *per capita* and consumer-only intakes of cranberry extract powder for specific demographic groups and for the total U.S. population. There were a number of assumptions included in the assessment which render exposure estimates that may be considered suitably conservative. For example, it has been assumed in both exposure assessments that all food products within a food category contain cranberry extract powder at the maximum specified level of use. In reality, the levels added to specific foods will vary depending on the nature of the food product and it is unlikely that cranberry extract powder will have 100% market penetration in all identified food categories.

In summary, on a consumer-only basis, the resulting mean and 90th percentile intakes of cranberry extract powder by the total U.S. population from all proposed food-uses, were estimated to be 332 mg/person/day (5.8 mg/kg body weight/day) and 639 mg/person/day (12.9 mg/kg body weight/day), respectively. Among the individual population groups, male teenagers were determined to have the greatest mean at 449 mg/person/day (6.4 mg/kg body weight/day), while male adults were determined to have the greatest 90th percentile consumer-only intakes of cranberry extract powder on an absolute basis at 856 mg/person/day (9.1 mg/kg body weight/day), respectively. Infants and young children had the lowest mean and 90th percentile consumer-only intakes of 242 and 486 mg/person/day, respectively. When intakes were expressed on a body weight basis, infants and young children had the highest mean and 90th percentile consumer-only intake of 20.0 and 35.2 mg/kg body weight/day, respectively.

On a consumer-only basis, the resulting mean and 90th percentile intakes of proanthocyanidins from cranberry extract powder by the total U.S. population from all proposed food-uses, were estimated to be 177 mg/person/day (3.1 mg/kg body weight/day) and 340 mg/person/day (6.9 mg/kg body weight/day), respectively. Among the individual population groups, male teenagers were determined to have the greatest mean at 239 mg/person/day (3.4 mg/kg body weight/day), while male adults were determined to have the greatest 90th percentile consumer-only intakes of proanthocyanidins from cranberry extract powder on an absolute basis at 456 mg/person/day (4.9 mg/kg body weight/day), respectively. When intakes were expressed on a body weight basis, infants and young children had the highest mean and 90th percentile consumer-only intake of 10.7 and 18.8 mg/kg body weight/day, respectively.

Among the total population (all ages), the mean and 90th percentile intakes of anthocyanins from cranberry extract powder were determined to be 27 mg/person/day (0.5 mg/kg body weight/day) and 51 mg/person/day (1.0 mg/kg body weight/day), respectively. The equivalent intakes of phenolics were 135 and 260 mg/person/day (2.4 and 5.3 mg/kg body weight/day), respectively; while organic acid intakes from this ingredient were 2.1 and 4.1 mg/person/day (0.04 and 0.08 mg/kg body weight/day), respectively.

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Appendix A
Estimated Daily Intake of Cranberry Extract Powder from Individual Proposed Food-Uses by Different Population Groups within the U.S. (2013-2014 NHANES Data)

Table A-1 Estimated Daily Intake of Cranberry Extract Powder from Individual Proposed Food-Uses by Infants and Young Children Aged Up to 2 Years within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/day)		Consumer-Only Intake (mg/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	**55**	**194**	**22.6**	**142**	**242**	**486**
Beverages and Beverage Bases							
Energy Drinks	0.0	0	0	0	0	0	0
Enhanced or Fortified Waters	0.6	<1*	na	0.4	1	75*	75*
Flavored or Carbonated Waters	1.5	1*	na	0.6	3	133*	186*
Sport and Electrolyte Drinks, Fluid Replacement Drinks	14.4	8*	na	5.0	26	156*	385*
Ready-to-Drink Cold Brew Coffee Drinks	0.0	0	0	0	0	0	0
Processed Fruits and Fruit Juices							
Fruit Drinks and Ades (Ready-to-Drink and Powder Mixes)	83.5	46	165	18.4	124	249	523

n = sample size; na = not available; NHANES = National Health and Nutrition Examination Surveys; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

Table A-2 Estimated Daily Intake of Cranberry Extract Powder from Individual Proposed Food-Uses by Children Aged 3 to 11 Years within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/day)		Consumer-Only Intake (mg/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	**147**	**407**	**55.6**	**721**	**263**	**518**
Beverages and Beverage Bases							
Energy Drinks	<0.1	<1*	na	0.1	1	116*	116*
Enhanced or Fortified Waters	0.5	1*	na	0.5	7	125*	190*
Flavored or Carbonated Waters	1.6	2	na	2.6	32	92	113*
Sport and Electrolyte Drinks, Fluid Replacement Drinks	14.5	21	58	12.2	127	174	339
Ready-to-Drink Cold Brew Coffee Drinks	<0.1	<1*	na	<0.1	1	48*	48*
Processed Fruits and Fruit Juices							
Fruit Drinks and Ades (Ready-to-Drink and Powder Mixes)	83.3	122	345	49.2	649	248	455

n = sample size; na = not available; NHANES = National Health and Nutrition Examination Surveys; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

Table A-3 Estimated Daily Intake of Cranberry Extract Powder from Individual Proposed Food-Uses by Female Teenagers Aged 12 to 19 Years within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/day)		Consumer-Only Intake (mg/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	**148**	**388**	**46.4**	**281**	**319**	**590**
Beverages and Beverage Bases							
Energy Drinks	<0.1	4*	na	1.9	7	194*	300*
Enhanced or Fortified Waters	2.7	4*	na	2.1	12	189*	262*
Flavored or Carbonated Waters	11.0	16*	na	4.1	16	398*	807*
Sport and Electrolyte Drinks, Fluid Replacement Drinks	12.7	19	na	9.2	55	204	368*
Ready-to-Drink Cold Brew Coffee Drinks	0.5	1*	na	1.1	5	74*	95*
Processed Fruits and Fruit Juices							
Fruit Drinks and Ades (Ready-to-Drink and Powder Mixes)	70.7	105	350	35.4	227	296	581

n = sample size; na = not available; NHANES = National Health and Nutrition Examination Surveys; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

Table A-4 Estimated Daily Intake of Cranberry Extract Powder from Individual Proposed Food-Uses by Male Teenagers Aged 12 to 19 Years within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/day)		Consumer-Only Intake (mg/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	**211**	**504**	**46.9**	**274**	**449**	**808**
Beverages and Beverage Bases							
Energy Drinks	<0.1	10*	na	4.0	8	241*	321*
Enhanced or Fortified Waters	0.8	2*	na	0.9	6	196*	225*
Flavored or Carbonated Waters	0.8	2*	na	1.7	5	98*	184*
Sport and Electrolyte Drinks, Fluid Replacement Drinks	41.2	87	233	19.8	92	439	620
Ready-to-Drink Cold Brew Coffee Drinks	0.6	1*	na	1.1	9	117*	140*
Processed Fruits and Fruit Juices							
Fruit Drinks and Ades (Ready-to-Drink and Powder Mixes)	52.0	110	310	29.8	205	368	755

n = sample size; na = not available; NHANES = National Health and Nutrition Examination Surveys; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

Table A-5 Estimated Daily Intake of Cranberry Extract Powder from Individual Proposed Food-Uses by Female Adults Aged 20 Years and Over within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/day)		Consumer-Only Intake (mg/day)			
		Mean	90th Percentile	%	n	Mean	
All	***100***	**100**	**317**	**31.5**	**719**	**316**	**634**
Beverages and Beverage Bases							
Energy Drinks	<0.1	2	na	1.3	31	178	316*
Enhanced or Fortified Waters	4.9	5	na	2.2	37	220	384*
Flavored or Carbonated Waters	13.6	14	na	5.7	98	236	478
Sport and Electrolyte Drinks, Fluid Replacement Drinks	8.3	8	na	3.3	73	248	388*
Ready-to-Drink Cold Brew Coffee Drinks	2.3	2	na	1.7	42	136	203*
Processed Fruits and Fruit Juices							
Fruit Drinks and Ades (Ready-to-Drink and Powder Mixes)	68.7	68	234	20.3	508	338	675

n = sample size; na = not available; NHANES = National Health and Nutrition Examination Surveys; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

Table A-6 Estimated Daily Intake of Cranberry Extract Powder from Individual Proposed Food-Uses by Male Adults Aged 20 Years and Over within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/day)		Consumer-Only Intake (mg/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	**113**	**350**	**30.1**	**673**	**375**	**856**
Beverages and Beverage Bases							
Energy Drinks	<0.1	9	na	4.2	90	217	310
Enhanced or Fortified Waters	3.6	4*	na	1.3	28	315*	477*
Flavored or Carbonated Waters	9.3	10	na	4.3	69	240	492*
Sport and Electrolyte Drinks, Fluid Replacement Drinks	21.3	24	na	8.0	162	300	600
Ready-to-Drink Cold Brew Coffee Drinks	1.2	1*	na	0.6	18	214*	222*
Processed Fruits and Fruit Juices							
Fruit Drinks and Ades (Ready-to-Drink and Powder Mixes)	56.5	64	233	17.1	407	372	726

n = sample size; na = not available; NHANES = National Health and Nutrition Examination Surveys; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

Table A-7 Estimated Daily Intake of Cranberry Extract Powder from Individual Proposed Food-Uses by the Total U.S. Population (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/day)		Consumer-Only Intake (mg/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	**116**	**354**	**35.1**	**2,810**	**332**	**639**
Beverages and Beverage Bases							
Energy Drinks	<0.1	5	na	2.3	137	210	322
Enhanced or Fortified Waters	3.2	4	na	1.5	91	240	475
Flavored or Carbonated Waters	8.7	10	na	4.4	223	232	492
Sport and Electrolyte Drinks, Fluid Replacement Drinks	17.3	20	na	7.3	535	277	469
Ready-to-Drink Cold Brew Coffee Drinks	1.3	1	na	1.0	75	149	203*
Processed Fruits and Fruit Juices							
Fruit Drinks and Ades (Ready-to-Drink and Powder Mixes)	65.4	76	262	23.7	2,120	321	634

n = sample size; na = not available; NHANES = National Health and Nutrition Examination Surveys; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

Appendix B
Estimated Daily Per Kilogram Body Weight Intake of Cranberry Extract Powder from Individual Proposed Food-Uses by Different Population Groups within the U.S. (2013-2014 NHANES Data)

Table B-1 Estimated Daily Per Kilogram Body Weight Intake of Cranberry Extract Powder from Individual Proposed Food-Uses by Infants and Young Children Aged Up to 2 Years within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	**4.5**	**15.6**	**22.7**	**142**	**20.0**	**35.2**
Beverages and Beverage Bases							
Energy Drinks	0.0	0	0	0	0	0	0
Enhanced or Fortified Waters	0.5	<0.1*	na	0.4	1	5.8*	5.8*
Flavored or Carbonated Waters	1.3	0.1*	na	0.6	3	9.7*	13.5*
Sport and Electrolyte Drinks, Fluid Replacement Drinks	14.3	0.7*	na	5.1	26	12.9*	31.9*
Ready-to-Drink Cold Brew Coffee Drinks	0.0	0	0	0	0	0	0
Processed Fruits and Fruit Juices							
Fruit Drinks and Ades (Ready-to-Drink and Powder Mixes)	83.8	3.8	12.4	18.5	124	20.6	40.5

bw = body weight; n = sample size; na = not available; NHANES = National Health and Nutrition Examination Surveys; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

Table B-2 Estimated Daily Per Kilogram Body Weight Intake of Cranberry Extract Powder from Individual Proposed Food-Uses by Children Aged 3 to 11 Years within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	
All	***100***	**5.3**	**15.1**	**55.5**	**716**	**9.5**	**18.1**
Beverages and Beverage Bases							
Energy Drinks	<0.1	<0.1*	na	0.1	1	3.4*	3.4*
Enhanced or Fortified Waters	0.5	<0.1*	na	0.5	7	5.2*	7.1*
Flavored or Carbonated Waters	1.7	0.1	na	2.7	32	3.3	5.3*
Sport and Electrolyte Drinks, Fluid Replacement Drinks	13.7	0.7	2.2	12.3	127	5.9	12.5
Ready-to-Drink Cold Brew Coffee Drinks	<0.1	<0.1*	na	<0.1	1	2.6*	2.6*
Processed Fruits and Fruit Juices							
Fruit Drinks and Ades (Ready-to-Drink and Powder Mixes)	84.0	4.5	13.4	48.9	644	9.1	17.5

bw = body weight; n = sample size; na = not available; NHANES = National Health and Nutrition Examination Surveys; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

Table B-3 Estimated Daily Per Kilogram Body Weight Intake of Cranberry Extract Powder from Individual Proposed Food-Uses by Female Teenagers Aged 12 to 19 Years within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	**2.4**	**6.6**	**46.3**	**277**	**5.1**	**10.6**
Beverages and Beverage Bases							
Energy Drinks	<0.1	0.1*	na	1.9	7	3.1*	5.7*
Enhanced or Fortified Waters	2.7	0.1*	na	2.1	12	3.1*	4.9*
Flavored or Carbonated Waters	10.6	0.3*	na	4.2	16	6.1*	12.2*
Sport and Electrolyte Drinks, Fluid Replacement Drinks	11.2	0.3	na	9.0	53	2.9	4.8*
Ready-to-Drink Cold Brew Coffee Drinks	0.6	<0.1*	na	1.1	5	1.3*	1.3*
Processed Fruits and Fruit Juices							
Fruit Drinks and Ades (Ready-to-Drink and Powder Mixes)	72.3	1.7	5.5	35.3	224	4.9	9.4

bw = body weight; n = sample size; na = not available; NHANES = National Health and Nutrition Examination Surveys; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

Table B-4 Estimated Daily Per Kilogram Body Weight Intake of Cranberry Extract Powder from Individual Proposed Food-Uses by Male Teenagers Aged 12 to 19 Years within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	**3.0**	**7.3**	**47.2**	**273**	**6.4**	**13.1**
Beverages and Beverage Bases							
Energy Drinks	<0.1	0.1*	na	4.0	8	2.1*	3.1*
Enhanced or Fortified Waters	0.8	<0.1*	na	0.9	6	2.9*	4.0*
Flavored or Carbonated Waters	1.1	<0.1*	na	1.7	5	1.9*	2.8*
Sport and Electrolyte Drinks, Fluid Replacement Drinks	38.1	1.2	2.5	19.9	92	5.8	10.7
Ready-to-Drink Cold Brew Coffee Drinks	0.6	<0.1*	na	1.2	9	1.6*	2.1*
Processed Fruits and Fruit Juices							
Fruit Drinks and Ades (Ready-to-Drink and Powder Mixes)	56.7	1.7	4.9	30.0	204	5.7	11.6

bw = body weight; n = sample size; na = not available; NHANES = National Health and Nutrition Examination Surveys; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

Table B-5 Estimated Daily Per Kilogram Body Weight Intake of Cranberry Extract Powder from Individual Proposed Food-Uses by Female Adults Aged 20 Years and Over within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	**1.3**	**4.3**	**31.6**	**716**	**4.2**	**8.8**
Beverages and Beverage Bases							
Energy Drinks	<0.1	<0.1	na	1.3	31	2.7	5.1*
Enhanced or Fortified Waters	4.7	0.1	na	2.2	37	2.8	4.6*
Flavored or Carbonated Waters	14.8	0.2	na	5.7	97	3.4	6.5
Sport and Electrolyte Drinks, Fluid Replacement Drinks	8.4	0.1	na	3.3	73	3.3	5.1*
Ready-to-Drink Cold Brew Coffee Drinks	2.0	<0.1	na	1.7	42	1.6	2.4*
Processed Fruits and Fruit Juices							
Fruit Drinks and Ades (Ready-to-Drink and Powder Mixes)	67.4	0.9	3.2	20.3	506	4.4	9.4

bw = body weight; n = sample size; na = not available; NHANES = National Health and Nutrition Examination Surveys; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

Table B-6 Estimated Daily Per Kilogram Body Weight Intake of Cranberry Extract Powder from Individual Proposed Food-Uses by Male Adults Aged 20 Years and Over within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	**1.3**	**4.2**	**30.2**	**671**	**4.3**	**9.1**
Beverages and Beverage Bases							
Energy Drinks	<0.1	0.1	na	4.3	90	2.5	3.8
Enhanced or Fortified Waters	3.4	<0.1*	na	1.3	28	3.4*	5.6*
Flavored or Carbonated Waters	9.3	0.1	na	4.4	69	2.7	5.5*
Sport and Electrolyte Drinks, Fluid Replacement Drinks	22.1	0.3	na	7.9	160	3.6	7.4
Ready-to-Drink Cold Brew Coffee Drinks	1.2	<0.1*	na	0.6	18	2.5*	3.0*
Processed Fruits and Fruit Juices							
Fruit Drinks and Ades (Ready-to-Drink and Powder Mixes)	55.7	0.7	2.7	17.3	407	4.2	8.3

bw = body weight; n = sample size; na = not available; NHANES = National Health and Nutrition Examination Surveys; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

Table B-7 Estimated Daily Per Kilogram Body Weight Intake of Cranberry Extract Powder from Individual Proposed Food-Uses by the Total U.S. Population (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	**2.0**	**5.9**	**35.1**	**2,795**	**5.8**	**12.9**
Beverages and Beverage Bases							
Energy Drinks	<0.1	0.1	na	2.3	137	2.5	4.3
Enhanced or Fortified Waters	2.4	<0.1	na	1.6	91	3.1	5.4
Flavored or Carbonated Waters	7.1	0.1	na	4.4	222	3.3	6.2
Sport and Electrolyte Drinks, Fluid Replacement Drinks	16.1	0.3	na	7.3	531	4.5	9.2
Ready-to-Drink Cold Brew Coffee Drinks	0.9	<0.1	na	1.0	75	1.8	2.5*
Processed Fruits and Fruit Juices							
Fruit Drinks and Ades (Ready-to-Drink and Powder Mixes)	70.6	1.4	4.6	23.7	2,109	6.1	13.7

bw = body weight; n = sample size; na = not available; NHANES = National Health and Nutrition Examination Surveys; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

Appendix C
Representative Food Codes for Proposed Food-Uses of Cranberry Extract Powder in the U.S. (2013-2014 NHANES Data)

Beverages and Beverage Bases

Energy Drinks

[Cranberry Extract Powder] = 62.5 mg/100 g

95310200	Full Throttle Energy Drink
95310400	Monster Energy Drink
95310500	Mountain Dew AMP Energy Drink
95310550	No Fear Energy Drink
95310555	No Fear Motherload Energy Drink
95310560	NOS Energy Drink
95310600	Red Bull Energy Drink
95310700	Rockstar Energy Drink
95310750	SoBe Energize Energy Juice Drink
95310800	Vault Energy Drink
95311000	Energy Drink
95312400	Monster Energy Drink, Lo Carb
95312500	Mountain Dew AMP Energy Drink, sugar-free
95312550	No Fear Energy Drink, sugar-free
95312555	NOS Energy Drink, sugar-free
95312560	Ocean Spray Cran-Energy Cranberry Energy Juice Drink
95312600	Red Bull Energy Drink, sugar-free
95312700	Rockstar Energy Drink, sugar-free
95312800	Vault Zero Energy Drink
95312900	XS Energy Drink
95312905	XS Gold Plus Energy Drink
95313200	Energy drink, sugar free

Enhanced or Fortified Waters

[Cranberry Extract Powder] = 62.5 mg/100 g

94210100	Propel Water
94210200	Glaceau Vitamin Water
94210300	SoBe Life Water
94220100	Propel Zero Water
94220110	Propel Zero Calcium Water
94220215	Glaceau Vitamin Water Zero
94220310	SoBe Life Water Zero

Flavored or carbonated water
[Cranberry Extract Powder] = 62.5 mg/100 g

92410110	Carbonated water, sweetened
92410210	Carbonated water, unsweetened
92410250	Carbonated water, sweetened, with low-calorie or no-calorie sweetener
94100300	Capri Sun Roarin' Waters
94100200	Water, bottled, sweetened, with low calorie sweetener

Sport or Electrolyte Drinks, Fluid Replacement Drinks
[Cranberry Extract Powder] = 62.5 mg/100 g

95320200	Gatorade G sports drink
95320500	Powerade sports drink
95321000	Sports drink, NFS
95322200	Gatorade G2 sports drink, low calorie
95322500	Powerade Zero sports drink, low calorie
95323000	Sports drink, low calorie
95330100	Fluid replacement, electrolyte solution
95330500	Fluid replacement, 5% glucose in water

Adjusted for not being reconstituted; reconstitution factor of 16.625
[Cranberry Extract Powder] = 1039.1 mg/100g

92900300	Sports drink, dry concentrate, not reconstituted

Ready-To-Drink Cold Brew Coffee Drinks
[Cranberry Extract Powder] = 62.5 mg/100 g

92102450	Iced Coffee, pre-lightened and pre-sweetened
92130000	Coffee, pre-lightened and pre-sweetened with sugar
92130001	Coffee, decaffeinated, pre-lightened and pre-sweetened with sugar
92130005	Coffee, pre-lightened and pre-sweetened with low calorie sweetener
92130006	Coffee, decaffeinated, pre-lightened and pre-sweetened with low calorie sweetener
92130010	Coffee, pre-lightened
92130011	Coffee, decaffeinated, pre-lightened
92130020	Coffee, pre-sweetened with sugar
92130021	Coffee, decaffeinated, pre-sweetened with sugar
92130030	Coffee, pre-sweetened with low calorie sweetener
92130031	Coffee, decaffeinated, pre-sweetened with low calorie sweetener
92171000	Coffee, bottled/canned
92171010	Coffee, bottled/canned, light

Processed Fruits and Fruit Juices

Fruit Drinks and Ades

[Cranberry Extract Powder] = 125 mg/100 g

92432000	Fruit juice drink, citrus, carbonated
92433000	Fruit juice drink, noncitrus, carbonated
92510610	Fruit juice drink
92510650	Tamarind drink (Refresco de tamarindo)
92510720	Fruit punch, made with fruit juice and soda
92510730	Fruit punch, made with soda, fruit juice, and sherbet or ice cream
92510955	Lemonade, fruit juice drink
92510960	Lemonade, fruit flavored drink
92511015	Fruit flavored drink
92511250	Fruit juice beverage, 40-50% juice, citrus
92512050	Frozen daiquiri mix, from frozen concentrate, reconstituted
92512090	Pina Colada, nonalcoholic
92512110	Margarita mix, nonalcoholic
92513000	Fruit flavored smoothie drink, frozen (no dairy)
92513010	Fruit flavored smoothie drink, frozen, light (no dairy)
92530410	Fruit flavored drink, with high vitamin C
92530510	Cranberry juice drink, with high vitamin C
92530610	Fruit juice drink, with high vitamin C
92530950	Vegetable and fruit juice drink, with high vitamin C
92531030	Sunny D
92541010	Fruit flavored drink, powdered, reconstituted
92542000	Fruit flavored drink, with high vitamin C, powdered, reconstituted
92550030	Fruit juice drink, with high vitamin C, light
92550035	Fruit juice drink, light
92550040	Fruit juice drink, diet
92550110	Cranberry juice drink, with high vitamin C, light
92550200	Grape juice drink, light
92550350	Orange juice beverage, 40-50% juice, light
92550360	Apple juice beverage, 40-50% juice, light
92550370	Lemonade, fruit juice drink, light
92550380	Pomegranate juice beverage, 40-50% juice, light
92550400	Vegetable and fruit juice drink, with high vitamin C, diet
92550405	Vegetable and fruit juice drink, with high vitamin C, light
92550610	Fruit flavored drink, with high vitamin C, diet
92550620	Fruit flavored drink, diet
92552000	Fruit flavored drink, with high vitamin C, powdered, reconstituted, diet
92552010	Fruit flavored drink, powdered, reconstituted, diet

92552020 Sunny D, reduced sugar
92552030 Capri Sun, fruit juice drink
92582100 Fruit juice drink, with high vitamin C, plus added calcium
92582110 Sunny D, added calcium

Adjusted for not being reconstituted; reconstitution factor of 4
[Cranberry Extract Powder] = 500 mg/100g

92511000 Lemonade, frozen concentrate, not reconstituted
92512040 Frozen daiquiri mix, frozen concentrate, not reconstituted

Adjusted for not being reconstituted; reconstitution factor of 10.23
[Cranberry Extract Powder] = 1,278.75 mg/100g

92900100 Fruit flavored drink, with high vitamin C, powdered, not reconstituted
92900110 Fruit flavored drink, powdered, not reconstituted
92900200 Fruit flavored drink, powdered, not reconstituted, diet

APPENDIX D
Expert Panel Statement

GRAS Panel Report Concerning the Generally Recognized as Safe (GRAS) Status of Cranberry Extract Powder for Use in Foods

14 May 2019

INTRODUCTION

At the request of Ocean Spray Cranberries, Inc. (hereafter Ocean Spray), Intertek convened a Panel (the "GRAS Panel") of independent scientists, qualified by their relevant national and international experience and scientific training to evaluate the safety of food ingredients, 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 nutrient in traditional foods, cranberry extract powder would be Generally Recognized as Safe (GRAS), based on scientific procedures. The GRAS Panel consisted of the below-signed qualified scientific experts: Dr. Joseph F. Borzelleca (Virginia Commonwealth University, School of Medicine), Dr. Robert J. Nicolosi (University of Massachusetts Lowell), and Dr. John A. Thomas (Indiana University School of Medicine).

The GRAS Panel, independently and collectively, critically evaluated a comprehensive package of publicly available scientific information and data compiled from the literature and other published sources based on searches of the published scientific literature conducted through January 2019. The GRAS Panel evaluated other information deemed appropriate or necessary, including data and information provided by Ocean Spray. The data evaluated by the GRAS Panel included the method of manufacture and product specifications, analytical data, intended use levels in specified food products, consumption estimates for all intended uses, and comprehensive literature on the safety of cranberry extract powder and its individual components.

Following independent, critical evaluation of such publicly available data and information, the GRAS Panel unanimously concluded that under the conditions of intended use in traditional foods described herein, cranberry extract powder, meeting appropriate food-grade specifications (see Table 1 in Appendix A), and manufactured in accordance with current good manufacturing practice, is GRAS based on scientific procedures. A summary of the basis for the GRAS Panel's conclusion is provided below.

COMPOSITION, MANUFACTURING, AND SPECIFICATIONS

Typically, cranberry juice contains sugars, organic acids, and phenolic compounds (including flavonols, flavan-3-ols, anthocyanins, and proanthocyanidins). Of the solid material in cranberry juice, 89.8% (±2.0%) are organic acids and sugars (Ocean Spray's internal compositional cranberry juice database). The organic acids and sugars are removed and phenolic compounds are selectively isolated from the other components of cranberry juice by Ocean Spray to yield cranberry extract powder. The phenolic compounds remain at the same levels and at the same relative concentrations as in 27% cranberry juice. The main components in cranberry extract powder include polyphenols, specifically proanthocyanidins (~55%), anthocyanins (5 to 8%), phenolics (35 to 38%), and organic acids (0.4 to 0.5%).

Food-grade cranberry juice concentrate is diluted from a product with a percentage soluble solids or standard degrees Brix of 50 to 25 degrees. The diluted concentrate is then loaded onto a column containing Amberlite™ XAD-7HP adsorbent resin (in the process step referred to as loading). After loading, a 5% ethanol wash is used to remove the sugars and organic acids from the resin column. The phenolic components are then eluted from the resin column using a 95% ethanol/5% water solvent mixture. The phenolic fraction is partially concentrated to 25 to 30% solids and the ethanol is recovered before the concentrated extract is spray dried to yield a powder. Silicon dioxide is added to the powder as a flow agent (0.6 to 0.8% of final product) and maltodextrin is added as a carrier (9 to 22% of final product). The manufacturing process is performed consistent with current Good Manufacturing Practice (cGMP). Analysis of 3 lots of cranberry extract power demonstrates that the manufacturing process produces a consistent product that meets specifications.

INTENDED USE AND ESTIMATED EXPOSURE

Cranberries and its polyphenolic components have a long history of safe consumption throughout the world. The daily background intake of flavonoids, particularly proanthocyanidins and anthocyanins within different food categories has been estimated by Scalbert and Williamson (2000) and Santos-Buelga and Scalbert (2000) to be in the range of 460 to 1,000 mg. Based on the high levels of anthocyanins present in fruits and vegetables, intakes of >100 mg/day could be achieved with regular consumption of select fruits or berries, such as blackberries, raspberries, blueberries, or Concord grapes (Wu *et al.*, 2006). Erdman *et al.* (2007) suggest a consumption of 58 mg proanthocyanidins per day for an average American consumer, while Wang *et al*. (2011) estimated proanthocyanidin intake at 95 mg/day.

Ocean Spray intends to market cranberry extract powder for use in beverages and beverage bases (*i.e*., energy drinks, enhanced or fortified waters, flavored or carbonated waters, ready-to-drink cold brew coffee drinks, sports and electrolyte drinks, and fluid replacement drinks) at a level of 150 mg/8 oz serving or 62.5 mg/100 g based on a serving size of 240 mL. Cranberry extract powder is proposed for use in processed fruits and fruit juices (*i.e.,* fruit drinks and ades [ready-to-drink and powder mixes]) at a level of 300 mg/8 oz serving or 125 mg/100 g, based on a serving size of 240 mL. These proposed uses of cranberry extract result in concentrations of these components comparable to what is found in 27% cranberry juice cocktail, but less than those found in 100% cranberry juice.

Exposure to cranberry extract powder based on the conditions of intended food-use is comparable to or lower than those in other foods. On a consumer-only basis, the resulting mean and 90th percentile intakes of cranberry extract powder from all proposed food-uses, were estimated to be 334 mg/person/day (5.9 mg/kg body weight/day) and 638 mg/person/day (13.0 mg/kg body weight/day), respectively. Mean and 90th percentile intakes of proanthocyanidins from all proposed food-uses of cranberry extract powder were estimated to be 178 mg/person/day (3.1 mg/kg body weight/day) and 340 mg/person/day (6.9 mg/kg body weight/day), respectively. Among the total population (all ages), the mean and 90th percentile intakes of anthocyanins from cranberry extract powder were determined to be 27 and 51 mg/person/day, respectively. The mean and 90th percentile intakes of phenolics among the total population (all ages) were determined to be 136 and 259 mg/person/day, respectively. The mean and 90th percentile intakes of organic acids from cranberry extract powder among the total population (all ages) were determined to be 2.1 and 4.1 mg/person/day, respectively. These levels and relative ratios of these components are comparable to those resulting from consumption of cranberry juice cocktail.

Comparison of the estimated levels of intake to the levels in the diet and the available published data demonstrate that the proposed use of cranberry extract powder in beverages described herein, is not expected to be a safety concern. Existing cranberry juice beverages available in the market contain much higher levels of polyphenols when compared to the level of polyphenols proposed for beverage use from cranberry extract powder. Existing cranberry juice beverages available in the market contain proanthocyanidins (*i.e*., 576 mg per serving), phenolics (*i.e.,* 736 mg per serving), and anthocyanins (*i.e*., 53 mg per serving), levels much greater than the levels proposed for beverage use from cranberry extract powder.

DATA PERTAINING TO SAFETY

The safety of cranberry extract powder is based on the long history of safe consumption of cranberries, cranberry juice, and cranberry juice cocktail at levels estimated from the proposed use of cranberry extract powder. This long history of safe consumption precluded the need for traditional assessments of safety.

The safety of various cranberry extracts was evaluated at an average dose of up to 0.60 mg/kg body weight/day of phenolic compounds following dietary administration to 10-week-old male Wistar rats for a period of 14 weeks (Palikova *et al*., 2010). The genotoxicity of cranberry extracts was evaluated in the Comet assay. Cranberry preparations have been used as an adjunctive treatment for urinary tract infections.

The safety of cranberry extract powder is also supported by the safety data available on its constituents (*e.g*., proanthocyanidins, anthocyanins, and other phenolics). Toxicity assessments of proanthocyanidin-rich grape seed extract include an acute toxicity study and 3 sub-chronic toxicity studies (two 90-day and one 6-month study) in mice and rats, and genotoxicity and mutagenicity studies (an Ames bacterial mutagenicity assay, an *in vitro* chromosome aberration assay in Chinese hamster lung cells, and an *in vivo* mouse micronucleus study).

Absorption, Distribution, Metabolism, and Excretion (ADME)

Proanthocyanidins and the anthocyanins, the primary polyphenols present in cranberry extract powder, are less bioavailable than other dietary polyphenols (*e.g*., isoflavones, catechins, flavanones). Differences in molecular size, interflavan linkages, and hydroxylation pattern on the constituent flavan-3-ols, influence the metabolism and biological effects of proanthocyanidins. Cranberry flavonol glycosides are absorbed into the circulatory system and conjugated to facilitate excretion. Urinary levels are higher than those in plasma, suggesting a lack of accumulation in organs or tissues. The polyphenols reaching the colon are extensively metabolized by the microflora into a wide array of low molecular weight phenolic acids. The extent and rate of absorption varies among individuals, likely due to individual differences in phase II enzyme polymorphisms as well as composition of gastrointestinal microbiota.

Toxicological Studies

Subchronic and Chronic Studies

Parameters relating to the safety of various cranberry extracts (*e.g*. food consumption, body weight, hematology, clinical chemistry, urinalysis, gross and histopathology) were reported by Palikova *et al.* (2010) following dietary administration to 10-week-old male Wistar rats for a period of 14 weeks and the no-observed-adverse-effect levels were the highest doses tested, 0.60 mg/kg body weight/day of phenolic compounds. There was no significant effect on any parameter evaluated was reported. No genotoxicity was reported when these extracts were evaluated in the comet assay. Although this study was not conducted with Ocean Spray's material *per se*, the test materials would have contained very similar polyphenolics relative to Ocean Spray's cranberry extract powder. Analysis of proanthocyanidin s in commercial products demonstrated that the Ocean Spray's cranberry extract powder is substantially chemically equivalent to the other cranberry extract products currently on the market. The results of Palikova *et al*. (2010) corroborate the safety of Ocean Spray's cranberry extract powder.

Mutagenicity and Genotoxicity

Palikova *et al*. (2010) reported on the potential genotoxicity of these substantially chemically equivalent cranberry powders by assessing the formation of single-stranded DNA breaks in peripheral lymphocytes in the Comet assay. Peripheral lymphocytes were isolated and DNA damage was analyzed using a fluorescence microscope, after staining with ethidium bromide. Analysis revealed no significant DNA damage or genotoxicity.

Clinical Studies

Information from clinical trials with cranberry extract powder and related cranberry products support the safety of Ocean Spray's ingredient under the proposed conditions of use in food. Clinical studies of Ocean Spray's cranberry extract powder demonstrate that it was well tolerated when consumed at doses up to 600 mg of proanthocyanidins/day in 2 divided doses for 12 weeks. The incidence of adverse effects in these studies were comparable to those seen in placebo and consisted of mainly gastrointestinal complaints, skin discoloration, headache, stuffy nose, frequent urination, and hyperactivity. In reported clinical studies of cranberry products in the treatment and prevention of urinary tract infections, only minor gastrointestinal side effects were reported.

SUMMARY

Ocean Spray manufactures cranberry extract powder from food-grade cranberry concentrate in accordance with cGMP. The organic acids and sugars typically found in cranberry juice are removed and phenolic compounds are selectively isolated. The main components in cranberry extract powder include polyphenols, specifically proanthocyanidins (~55%), anthocyanins (5 to 8%), phenolics (35 to 38%), and organic acids (0.4 to 0.5%). Microbiological and chemical analyses demonstrated that cranberry extract powder conforms to product specifications. Cranberry extract powder is stable for several years when kept under dry conditions at room temperature and below.

Ocean Spray intends to market cranberry extract powder for use in beverages and beverage bases (*i.e*., energy drinks, enhanced or fortified waters, flavored or carbonated waters, ready-to-drink cold brew coffee drinks, sports and electrolyte drinks, and fluid replacement drinks) at a level of 150 mg/8 oz serving or 62.5 mg/100 g based on a serving size of 240 mL [8 oz] These proposed uses of cranberry extract powder result in concentrations of proanthocyanidins comparable to what is found in 27% cranberry juice cocktail, but less than those found in 100% cranberry juice.

Cranberries and cranberry juice have a long history of safe consumption throughout the world. The natural presence of polyphenols in berries, fruits, and vegetables results in background intakes comparable to or greater than that resulting from proposed uses. Published preclinical safety data for cranberry extracts and related polyphenolic rich extracts and published clinical studies with cranberry juice and extracts corroborate the safety of Ocean Spray's cranberry extract powder.

In summary, a critical evaluation of the manufacturing, estimates of consumer exposure, and safety and clinical data on the polyphenolic constituents of cranberry extract powder supports the conclusion that Ocean Spray's cranberry extract powder can be safely used under the proposed conditions of use.

CONCLUSION

We, the Expert Panel, have, independently and collectively, critically evaluated the data and information summarized above and conclude that cranberry extract powder, meeting appropriate food-grade specifications and produced in according with current Good Manufacturing Practice, is Generally Recognized as Safe (GRAS) based on scientific procedures under the conditions of intended use in foods specified herein. It is our professional opinion that other qualified experts would also concur in this conclusion.

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APPENDIX A
Product Specifications

Table 1 Chemical and Microbiological Specifications for Cranberry Extract Powder

Specification Parameter	Specification
Moisture (% w/w)	≤ 5
PACS (% dwb) OSC-DMAC Method BL-DMAC Method	 55.0–60.0 15.0–18.0
Total phenolics (GAE, % dwb)	< 46.2
Bulk density (loose) (g/mL or g/cm^3)	Read & Record
Solubility	100%, with no visible insoluble particles
Ethanol content (ppm)	≤ 100
Arsenic (ppm)	< 3
Lead (ppm)	< 2
Cadmium (ppm)	< 10
Mercury (ppm)	< 1
Screen analysis	100% through 30 mesh screen Read & Record % on 30, 60, 100, 140, 200, 325 mesh, and in pan.
Appearance & aroma; as powder	Free-flowing; deep red color. Earthy aroma with no burnt character
Appearance & aroma; as beverage	Crystal clear, pink/red color; bland flavor with slight astringency but no off flavors
Yeast	< 100 CFU/g
Mold	< 100 CFU/g
Aerobic Plate Count	< 1,000 CFU/g
Coliforms	< 3 MPN/g or <10 CFU/g
Escherichia coli	< 3 MPN/g or <10 CFU/g

CFU = colony forming units; dwb = dry weight basis; GAE = gallic acid equivalents; MPN = most probable number; ppm = parts per million.