

## GRAS Notice for Hydrolyzed Porcine Trachea Cartilage

***Prepared for:*** Office of Food Additive Safety (FHS-200)  
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Food and Drug Administration  
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May 3, 2017

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# GRAS Notice for Hydrolyzed Porcine Trachea Cartilage

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# GRAS Notice for Hydrolyzed Porcine Trachea Cartilage

## Part 1. §170.225 Signed Statements and Certification

In accordance with 21 CFR §170 Subpart E consisting of §170.203 through 170.285, Rousselot BVBA (Rousselot) hereby informs the U.S. Food and Drug Administration (FDA) of the view that hydrolyzed porcine cartilage, manufactured by Rousselot under the trade name Peptan II, is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act. This is based on Rousselot's conclusion that the notified substance is Generally Recognized as Safe (GRAS) under the conditions of its intended use described in Part 1.3 below. In addition, as a responsible official of Rousselot, Fred Beekmans hereby certifies that all data and information presented in this notice constitutes a complete, representative, and balanced submission which considered all unfavorable, as well as favorable, information known to Rousselot and pertinent to the evaluation of the safety and GRAS status of Peptan II as an ingredient for addition to food, as described herein.

Signed,

(b) (6)

(b) (6)

May 30, 2017

Date

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### 1.1 Name and Address of Notifier

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<sup>1</sup> Darling Ingredients consists of several business units, including Rousselot.

## 1.2 Common Name of Notified Substance

Hydrolyzed porcine trachea cartilage

## 1.3 Conditions of Use

Peptan II is intended for addition to a variety of food products intended for consumption by adults [including active nutrition and nutritionally complete bars, granola bars, enhanced fortified water beverages, sports nutrition gels, fortified flavored milk beverages (excluding milkshakes), enhanced or fortified fruit-flavored beverages, and gummies] at use levels of up to 5.0%. The intended food categories and use levels at which Rousselot's Peptan II will be added are summarized in Table 1.3-1.

| Food Category                     | Proposed Food-Uses  | Proposed Use Level of Peptan (g/RACC) | RACC* (g/mL)           | Peptan Use Level (%) <sup>a</sup> |
|-----------------------------------|---|---------------------------------------|------------------------|-----------------------------------|
| Baked Goods and Baking Mixes      | Active Nutrition and Nutritionally Complete Bars and Granola Bars | 1.2                                   | 40                     | 3.0                               |
| Beverage and Beverage Bases       | Enhanced Fortified Water Beverages                                | 2.0                                   | 240                    | 0.83                              |
|                                   | Sports Nutrition Gels   | 2.0                                   | 40 (360 <sup>b</sup> ) | 0.56                              |
| Milk Products                     | Fortified Flavored Milk Beverages (Excluding Milkshakes)          | 2.0                                   | 240                    | 0.83                              |
| Processed Fruits and Fruit Juices | Enhanced or Fortified Fruit-Flavored Beverages                    | 2.0                                   | 240                    | 0.83                              |
| Soft Candy                        | Gummies <sup>c</sup>  | 1.2                                   | 30                     | 5.0                               |

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

\* Serving sizes based on the US FDA Reference Amounts Customarily Consumed per Eating Occasion (RACCs) (U.S. FDA, 2016b).

<sup>a</sup> Peptan use level (%) adjusted according to RACC.

<sup>b</sup> No food codes were identified in the NHANES 2011-2012 dataset for sports gels, however based on the similar pattern of consumption, sports drinks were selected as a surrogate. As such, while the average serving size for a sports gel is approximately 40 g, the use level considered in the exposure assessment [*i.e.*, expressed on a percent (%) basis] was calculated based on a serving size of a sports drink (*i.e.*, 360 ml).

<sup>c</sup> As there were no food codes identified for gummies in the NHANES 2011-2012 dataset, food codes for gummy-like and fruit-snack type candies were selected as surrogates.

## 1.4 Basis for GRAS

Pursuant to 21 CFR § 170.30 (a) and (b) of the *Code of Federal Regulations* (CFR), hydrolyzed porcine cartilage (Peptan II) manufactured by Rousselot, has been concluded to have GRAS status for use as an ingredient for addition to specified conventional food and beverage products, as described in Part 1.3, 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 made available to the FDA for review and copying upon request during business hours at the offices of:

Darling Ingredients  
Kanaaldijk Noord 20-21  
5691 NM Son  
The Netherlands

In addition, should the FDA have any questions or additional information requests regarding this notification during or after the Agency's review of the notice, Rousselot will supply these data and information.

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

It is Rousselot'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 exempt from the Freedom of Information Act, 5 U.S.C. Section 552.

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

### 2.1 Identity

Peptan II is derived from porcine trachea cartilage, and contains primarily 2 different, unbound components (*i.e.*, ≥60% type II collagen and 10 to 25% chondroitin sulfate), both of which are endogenous to humans and have long histories of dietary consumption.

### 2.2 Method of Manufacturing

#### 2.2.1 Raw Materials and Processing Aids

Peptan II is derived from porcine trachea cartilage using an enzymatic hydrolysis process. The porcine trachea cartilage raw material used in the production of Peptan II is obtained from healthy animals in a food-grade manner as approved by competent European Union (EU) authorities.

The processing aids used in the manufacture of Peptan II are suitable for use in the production of food and conform to appropriate federal regulations pertaining to their use in food production.

## 2.2.2 Manufacturing Process

Peptan II is produced by enzymatic hydrolysis of porcine trachea cartilage, which is sourced from healthy animals in a food-grade manner as approved by competent EU authorities. Residual meat is removed from the cartilage prior to cutting, treatment with hydrogen peroxide (if required), and washing with water. The pH of the solution is adjusted as necessary. Using only water as a solvent, enzymatic hydrolysis is carried out. The hydrolysis residue (consisting of non-hydrolyzed cartilage and meat, fat, and bone residues) is then discarded. The remaining solution is then heated to inactivate any residual hydrolysis enzyme. The solution is then cooled, filtered, and concentrated. This solution is then sterilized and spray-dried.

## 2.2.3 Quality Control

In the EU, all food manufacturers need a mandatory Food Safety system compliant with Hazard Analysis and Critical Control Points (HACCP) principles, which is monitored by competent authorities (Regulation EU 852/2004 – EC, 2004a).

Peptan II complies with requirements for viral safety. Peptan II is manufactured from porcine cartilage from animals deemed fit for human consumption<sup>2</sup>, and sourced from establishments registered according to European regulations for porcine food products. The manufacturing process for Peptan II includes various steps which are known to inactivate any viruses that may be present in the starting material, including treatment with hydrogen peroxide, hydrolysis, heating to inactivate any residual enzyme, filtration and concentration, and sterilization. These steps ensure that the final Peptan II product is free of any residual enzyme or microbes.

## 2.3 Product Specifications and Batch Analyses

### 2.3.1 Product Specifications

Appropriate food-grade product specifications have been established for Rousselot's Peptan II and are presented in Table 2.3.1-1. Rousselot is a member of the "Gelatine Manufacturers of Europe" (GME) organization. All GME members are obliged to use the standardized GME methods for the testing of edible gelatin; Rousselot has accordingly adopted these specification parameters and test methods for their Peptan II product. Thus, Peptan II meets the relevant specifications of the GME. All methods of analysis are internationally recognized or are in-house methods that have been validated by Rousselot.

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<sup>2</sup> This is determined by EU regulation. Cartilage is mentioned in EU regulation 853/2004, section XVI (EC, 2004b): *The raw materials used for the manufacturing of the highly refined products referred to in point 1 must derive from: (a) animals, including feathers thereof, which have been slaughtered in a slaughterhouse and whose carcasses have been found fit for human consumption following ante-mortem and post-mortem inspection.* The competent veterinary authority monitors the food grade sourcing of raw materials.

**Table 2.3.1-1 Chemical and Microbiological Specifications for Peptan II**

| Specification Parameter           | Specification        | Method                      |
|-----------------------------------|----------------------|-----------------------------|
| <b>Identification</b>             |                      |                             |
| Mean molecular weight             | 2,000 to 6,000 g/mol | Rousselot <sup>a</sup>      |
| Collagen content                  | ≥60%                 | GME                         |
| Chondroitin sulfate               | 10 to 25%            | Rousselot by HPLC           |
| Hyaluronic acid                   | 0.1 to 1.0%          | External by HPLC            |
| pH (55°C, 6.67%)                  | 5.0 to 6.5           | GME                         |
| Viscosity (20%, 25°C)             | 8 to 15 mPa*s        | GME                         |
| Color (6.67%)                     | ≤6.0 Helliges        | Rousselot (internal method) |
| Loss on drying (105°C, 17 h)      | ≤12%                 | GME                         |
| Residue on ignition (550°C)       | ≤10%                 | GMIA                        |
| <b>Impurities</b>                 |                      |                             |
| Arsenic                           | ≤0.1 ppm             | GME                         |
| Cadmium                           | ≤0.5 ppm             | GME                         |
| Chromium                          | ≤10 ppm              | GME                         |
| Copper                            | ≤30 ppm              | GME                         |
| Mercury                           | ≤0.1 ppm             | GME                         |
| Lead                              | ≤1.0 ppm             | GME                         |
| Zinc                              | ≤50 ppm              | GME                         |
| Sulfites (SO <sub>2</sub> )       | ≤10 ppm              | GME                         |
| Peroxides                         | ≤10 ppm              | GME                         |
| <b>Microbiological Parameters</b> |                      |                             |
| Total aerobic microbial count     | ≤1,000 CFU/g         | GME                         |
| Total yeasts and molds count      | ≤100 CFU/g           | EP/USP                      |
| <i>Escherichia coli</i>           | Absent in 10 g       | GME                         |
| <i>Salmonella</i>                 | Absent in 25 g       | GME                         |
| Anaerobic sulfite-reducing spores | ≤10 CFU/g            | GME                         |

CFU = colony-forming units; EP = European Pharmacopoeia; GME = Gelatin Manufacturers of Europe; GMIA = Gelatin Manufacturers Institute of America; HPLC = high-performance liquid chromatography; USP = United States Pharmacopoeia.

<sup>a</sup> Determination of the mean molecular weight (Mw) was performed using high pressure size exclusion chromatography (HP-SEC). Samples were dissolved and diluted in ultrapure water to obtain a 0.2% solution, and injected in an HPLC system with TSKgel G2000 SWXL column and TSKgel SWXL guard column (Tosoh BioScience) at 30°C, with UV detection at 214 nm. A 0.2 M NaCl, 0.2 M NaH<sub>2</sub>PO<sub>4</sub>.H<sub>2</sub>O:acetonitrile (85:15) buffer at pH 5.3 was used at a flow rate of 0.5 ml/min to elute the samples. For calibration, collagen fragments between 210 and 38,000 Da were used.

### 2.3.2 Batch Analyses

Three non-consecutive batches of Peptan II were analyzed to verify that the manufacturing process produces a consistent product that meets the proposed product specifications.

Analysis of sample lots of Peptan II, as provided by Rousselot, demonstrates that the manufacturing process as described in Part 2.2.2 results in a consistent product compliant with the specifications and additional compositional parameters of the GME.

### 2.3.3 Additional Analytical Information

#### 2.3.3.1 Amino Acid Profile

The average amino acid content of Peptan II was calculated using the results of the analysis of 2 batches of Peptan II, and is presented in Table 2.3.3.1-1.

| <b>Table 2.3.3.1-1 Amino Acid Content of Peptan II</b> |                                     |
|--|-------------------------------------|
| <b>Amino Acid</b>                                      | <b>Percent content in Peptan II</b> |
| Hydroxyproline   | 8.95                                |
| Aspartate  | 5.40                                |
| Serine   | 3.90                                |
| Glutamate  | 8.40                                |
| Glycine  | 29.85                               |
| Histidine  | 0.36                                |
| Arginine   | 4.80                                |
| Threonine  | 2.30                                |
| Alanine  | 9.80                                |
| Proline  | 11.65                               |
| Cysteine   | ND                                  |
| Tyrosine   | 0.32                                |
| Hydroxylysine  | 1.30                                |
| Valine   | 3.10                                |
| Methionine   | 0.805                               |
| Lysine   | 2.90                                |
| Isoleucine   | 1.55                                |
| Leucine  | 3.20                                |
| Phenylalanine  | 1.50                                |

### 2.4 Stability

Accelerated and real-time stability studies have been conducted on 3 non-consecutive batches of Peptan II. Peptan II (stored in paper bags with polyethylene liners) is stable for at least 6 months under standardized conditions (25°C and 60% relative humidity) and at least 12 months under warehouse (ambient) conditions. Although variations in color and moisture content were reported upon storage of Peptan II under accelerated storage conditions (40°C and 75% relative humidity), measurements of collagen and chondroitin sulfate remained within the defined limits. Together, these data support the proposed shelf-life for Peptan II of 12 months.

## Part 3. §170.235 Dietary Exposure

### 3.1 Current Regulatory Status of Similar Cartilage-Derived Products, Collagen Peptides, and Chondroitin Sulfate

Peptan II is a product containing primarily 2 different, unbound components with different structures/chemistry (*i.e.*, type II collagen and chondroitin sulfate). A commercial product similar to Peptan II (BioCell Collagen<sup>3</sup>, comprising 60% type II collagen, 20% chondroitin sulfate, and 10% hyaluronic acid) has been self-determined to be GRAS based on scientific procedures (BioCell Technology, LLC, 2017a). Other commercial products that are also similar to Peptan II (*i.e.*, extracts of connective tissue consisting of collagen and/or chondroitin sulfate, including Summit Nutritionals Cartilage Powder [Summit Nutritionals International, Inc., 2012]<sup>4</sup>, and Copalis Collagen Type II/Cartilage products [Copalis, 2017]<sup>5</sup>) are currently marketed in the U.S. as dietary supplements and/or food ingredients.

Peptones (*i.e.*, mixtures of polypeptides, oligopeptides, and amino acids produced by hydrolysis or denaturation of proteins, including animal tissue and gelatin) are GRAS for use in foods (21 CFR §184.1553) (U.S. FDA, 2016a). Gelatin (or hydrolyzed collagen, a high-molecular weight peptide obtained from hydrolysis of unspecified molecular types of collagen) and hydrolyzed gelatin have long histories of safe use in foods, supplements, and in pharmaceutical dosage capsules (Moskowitz, 2000), and gelatin is considered to be a food in the U.S. (21 CFR §170.3) (U.S. FDA, 2016a). Gelatin is permitted for use as a direct food additive used for microencapsulation of flavoring substances (21 CFR §172.230) (U.S. FDA, 2016a), and is GRAS for use as a food substance [Select Committee on GRAS Substances (SCOGS) Report No. 58, 1975] (FASEB, 1975). The FDA had no questions in response to a GRAS notification for pork collagen (molecular type not specified) when used as a binder or purge-reducing additive in meat or meat-type products at levels of 1 to 3.5% (U.S. FDA, 1999).

Chondroitin sulfate (from multiple sources) is widely used in dietary supplements worldwide. In the U.S., chondroitin sulfate is included on the list of dietary ingredients “grandfathered” under the *Dietary Supplement Health and Education Act of 1994* (DSHEA, 1994) prepared by the Council for Responsible Nutrition (CRN) and the United Natural Products Alliance (UNPA) (CRN, 1998; UNPA, 1999). The FDA had no questions in response to a GRAS notification for chondroitin sulfate sodium intended for use as an ingredient in foods providing up to 1,200 mg/day (GRN 666) (U.S. FDA, 2017). In Canada, there is a Natural Health Product (NHP) monograph for chondroitin sulfate, in which it is indicated that chondroitin sulfate may be used as a medicinal ingredient at doses of 800 to 1,200 mg/day (Health Canada, 2008).

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<sup>33</sup> <http://www.biocellcollagen.com/>

<sup>4</sup> <http://summitnutritionals.com/>

<sup>5</sup> <http://www.copalis.fr/>

Chondroitin sulfate also has a long history of use in food supplements in the EU at use levels providing up to 1,200 mg/day (Boots UK Limited, 2017; Holland & Barrett Retail Limited, 2017; Vitabiotics Ltd., 2017)<sup>6</sup>.

## **3.2 Probable Consumption**

An assessment of the anticipated dietary exposure to Peptan II as an ingredient under the intended conditions of use (see Table 1.3-1) was conducted using the most current data available at the time of analysis in the 2011-2012 cycles of the U.S. National Center for Health Statistics' (NCHS) National Health and Nutrition Examination Survey (NHANES) (CDC, 2015). The estimated daily intakes for Peptan II under its intended conditions of use are presented in Part 3.2.1 below. An assessment of the estimated daily intake for the individual components of Peptan II, namely type II collagen and chondroitin sulfate, was also conducted, as described below in Part 3.2.2 and Part 3.2.3, respectively.

### **3.2.1 Dietary Intake in General U.S. Population from all Proposed Food Uses – Peptan II**

A summary of the estimated daily intake of Peptan II from proposed food-uses is provided in Table 3.2.1-1 on an absolute basis (g/person/day), and in Table 3.2.1-2 on a body weight basis (mg/kg body weight/day).

The percentage of users was relatively low among all age groups evaluated in the current intake assessment. A range of 25.0 to 58.6% of the population groups consisted of “users” of those food products in which Peptan II is currently proposed for use. The all-user intakes are more applicable to the assessment of safety as they are more likely to represent exposure in the target populations. Consequently, only the all-user intake results will be discussed in detail.

Among the total population, the mean and 90<sup>th</sup> percentile all-user intakes of Peptan II were determined to be 1.8 and 4.1 g/person/day, respectively. Of the individual population groups, male teenagers and male adults were both determined to have the greatest mean all-user intake of Peptan II on an absolute basis, at 2.3 g/person/day, while male teenagers were determined to have the highest 90<sup>th</sup> percentile intake of Peptan II at 4.9 g/person/day. Infants and young children had the lowest mean and 90<sup>th</sup> percentile all-user intakes of 1.3 and 2.7 g/person/day, respectively (Table 3.2.1-1).

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<sup>6</sup> <http://www.boots.com/webapp/wcs/stores/servlet/EndecaSearchListView?storeId=10052&langId=-1&catalogId=10551&stReg=1&searchTerm=chondroitin+sulphate&newDepSearch=&x=0&y=0#container>  
<http://www.vitabiotics.com/jointace/max>  
<http://www.hollandandbarrett.com/shop/product/holland-barrett-max-strength-glucosamine-chondroitin-caplets-60041631>

**Table 3.2.1-1 Summary of the Estimated Daily Intake of Peptan II from Proposed Food-Uses in the U.S. by Population Group (2011-2012 NHANES Data)**

| Population Group           | Age Group (Years) | All-Person Consumption (g/day) |                             | All-Users Consumption (g/day) |       |      |                             |
|----------------------------|-------------------|--------------------------------|-----------------------------|-------------------------------|-------|------|-----------------------------|
|                            |                   | Mean                           | 90 <sup>th</sup> Percentile | % Users                       | n     | Mean | 90 <sup>th</sup> Percentile |
| Infants and Young Children | 0 to 2            | 0.4                            | 1.5                         | 34.9                          | 220   | 1.3  | 2.7                         |
| Children                   | 3 to 11           | 0.9                            | 2.4                         | 58.6                          | 890   | 1.5  | 3.0                         |
| Female Teenagers           | 12 to 19          | 0.9                            | 2.7                         | 44.4                          | 251   | 2.0  | 4.8                         |
| Male Teenagers             | 12 to 19          | 1.0                            | 3.5                         | 44.6                          | 235   | 2.3  | 4.9                         |
| Female Adults              | 20 and up         | 0.4                            | 1.3                         | 25.0                          | 548   | 1.5  | 3.4                         |
| Male Adults                | 20 and up         | 0.7                            | 2.2                         | 28.1                          | 539   | 2.3  | 4.7                         |
| Total Population           | All Ages          | 0.6                            | 2.0                         | 32.7                          | 2,683 | 1.8  | 4.1                         |

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

On a body weight basis, the total population mean and 90<sup>th</sup> percentile all-user intakes of Peptan II were determined to be 36.5 and 80.2 mg/kg body weight/day, respectively. Among the individual population groups, infants and young children were identified as having the highest mean and 90<sup>th</sup> percentile all-user intakes of any population group, of 100.0 and 221.0 mg/kg body weight/day, respectively<sup>7</sup>. Female adults had the lowest mean and 90<sup>th</sup> percentile all-user intakes of 20.9 and 46.2 mg/kg body weight/day, respectively (Table 3.2.1-2).

**Table 3.2.1-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Peptan II from Proposed Food-Uses in the U.S. by Population Group (2011-2012 NHANES Data)**

| Population Group           | Age Group (Years) | All-Person Consumption (mg/kg bw/day) |                             | All-Users Consumption (mg/kg bw/day) |       |       |                             |
|----------------------------|-------------------|---------------------------------------|-----------------------------|--------------------------------------|-------|-------|-----------------------------|
|                            |                   | Mean                                  | 90 <sup>th</sup> Percentile | %                                    | n     | Mean  | 90 <sup>th</sup> Percentile |
| Infants and Young Children | 0 to 2            | 35.1                                  | 116.0                       | 34.9                                 | 219   | 100.0 | 221.0                       |
| Children                   | 3 to 11           | 34.6                                  | 90.6                        | 58.6                                 | 890   | 59.0  | 114.0                       |
| Female Teenagers           | 12 to 19          | 15.0                                  | 47.0                        | 44.6                                 | 245   | 33.7  | 69.4                        |
| Male Teenagers             | 12 to 19          | 16.5                                  | 54.8                        | 44.8                                 | 235   | 37.0  | 85.9                        |
| Female Adults              | 20 and up         | 5.2                                   | 17.1                        | 24.9                                 | 540   | 20.9  | 46.2                        |
| Male Adults                | 20 and up         | 7.7                                   | 25.3                        | 28.2                                 | 533   | 27.2  | 57.9                        |
| Total Population           | All Ages          | 12.0                                  | 38.5                        | 32.7                                 | 2,662 | 36.5  | 80.2                        |

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

<sup>7</sup> Rousselot has confirmed that Peptan II is intended for addition to foods intended to be consumed by adults only.

### 3.2.2 Dietary Intake in General U.S. Population from all Proposed Food Uses – Type II Collagen

The calculations presented in this section are based on the average level of 73% type II collagen in Peptan II (see Table 2.3.1-1).

Among the total population, the mean and 90<sup>th</sup> percentile all-user intakes of type II collagen were determined to be 1.3 and 3.0 g/person/day, respectively. Of the individual population groups, male teenagers and male adults were both determined to have the greatest mean all-user intake of type II collagen on an absolute basis, at 1.7 g/person/day, while male teenagers were determined to have the highest 90<sup>th</sup> percentile intake of type II collagen at 3.6 g/person/day (Table 3.2.2-1).

| Population Group           | Age Group (Years) | All-Person Consumption (g/day) |                             | All-Users Consumption (g/day) |       |      |                             |
|----------------------------|-------------------|--------------------------------|-----------------------------|-------------------------------|-------|------|-----------------------------|
|                            |                   | Mean                           | 90 <sup>th</sup> Percentile | % Users                       | n     | Mean | 90 <sup>th</sup> Percentile |
| Infants and Young Children | 0 to 2            | 0.3                            | 1.1                         | 34.9                          | 220   | 0.9  | 2.0                         |
| Children                   | 3 to 11           | 0.6                            | 1.7                         | 58.6                          | 890   | 1.1  | 2.2                         |
| Female Teenagers           | 12 to 19          | 0.6                            | 2.0                         | 44.4                          | 251   | 1.5  | 3.5                         |
| Male Teenagers             | 12 to 19          | 0.7                            | 2.5                         | 44.6                          | 235   | 1.7  | 3.6                         |
| Female Adults              | 20 and up         | 0.3                            | 1.0                         | 25.0                          | 548   | 1.1  | 2.5                         |
| Male Adults                | 20 and up         | 0.5                            | 1.6                         | 28.1                          | 539   | 1.7  | 3.4                         |
| Total Population           | All Ages          | 0.4                            | 1.5                         | 32.7                          | 2,683 | 1.3  | 3.0                         |

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

On a body weight basis, the total population mean and 90<sup>th</sup> percentile all-user intakes of type II collagen were determined to be 26.6 and 58.5 mg/kg body weight/day, respectively. Among the individual population groups, infants and young children were identified as having the highest mean and 90<sup>th</sup> percentile all-user intakes of any population group, of 73.0 and 161.3 mg/kg body weight/day, respectively (Table 3.2.2-2).

**Table 3.2.2-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Type II Collagen from Proposed Food-Uses of Peptan II in the U.S. by Population Group (2011-2012 NHANES Data)**

| Population Group           | Age Group (Years) | All-Person Consumption (mg/kg bw/day) |                             | All-Users Consumption (mg/kg bw/day) |       |      |                             |
|----------------------------|-------------------|---------------------------------------|-----------------------------|--------------------------------------|-------|------|-----------------------------|
|                            |                   | Mean                                  | 90 <sup>th</sup> Percentile | %                                    | n     | Mean | 90 <sup>th</sup> Percentile |
| Infants and Young Children | 0 to 2            | 25.6                                  | 84.7                        | 34.9                                 | 219   | 73.0 | 161.3                       |
| Children                   | 3 to 11           | 25.3                                  | 66.1                        | 58.6                                 | 890   | 43.1 | 83.2                        |
| Female Teenagers           | 12 to 19          | 11.0                                  | 34.3                        | 44.6                                 | 245   | 24.6 | 50.7                        |
| Male Teenagers             | 12 to 19          | 12.0                                  | 40.0                        | 44.8                                 | 235   | 27.0 | 62.7                        |
| Female Adults              | 20 and up         | 3.8                                   | 12.5                        | 24.9                                 | 540   | 15.3 | 33.7                        |
| Male Adults                | 20 and up         | 5.6                                   | 18.5                        | 28.2                                 | 533   | 19.9 | 42.3                        |
| Total Population           | All Ages          | 8.8                                   | 28.1                        | 32.7                                 | 2,662 | 26.6 | 58.5                        |

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

### 3.2.3 Dietary Intake in General U.S. Population from all Proposed Food Uses – Chondroitin Sulfate

The calculations presented in this section are based on the maximum level of 25% chondroitin sulfate in Peptan II (see specification limit in Table 2.3.1-1).

Among the total population, the mean and 90<sup>th</sup> percentile all-user intakes of chondroitin sulfate were determined to be 0.5 and 1.0 g/person/day, respectively. Of the individual population groups, male teenagers and male adults were both determined to have the greatest mean all-user intake of chondroitin sulfate on an absolute basis, at 0.6 g/person/day, while male and female teenagers, and male adults were all determined to have the highest 90<sup>th</sup> percentile intake of chondroitin sulfate at 1.2 g/person/day (Table 3.2.3-1).

**Table 3.2.3-1 Summary of the Estimated Daily Intake of Chondroitin Sulfate from Proposed Food-Uses of Peptan II in the U.S. by Population Group (2011-2012 NHANES Data)**

| Population Group           | Age Group (Years) | All-Person Consumption (g/day) |                             | All-Users Consumption (g/day) |     |      |                             |
|----------------------------|-------------------|--------------------------------|-----------------------------|-------------------------------|-----|------|-----------------------------|
|                            |                   | Mean                           | 90 <sup>th</sup> Percentile | % Users                       | n   | Mean | 90 <sup>th</sup> Percentile |
| Infants and Young Children | 0 to 2            | 0.1                            | 0.4                         | 34.9                          | 220 | 0.3  | 0.7                         |
| Children                   | 3 to 11           | 0.2                            | 0.6                         | 58.6                          | 890 | 0.4  | 0.8                         |
| Female Teenagers           | 12 to 19          | 0.2                            | 0.7                         | 44.4                          | 251 | 0.5  | 1.2                         |
| Male Teenagers             | 12 to 19          | 0.3                            | 0.9                         | 44.6                          | 235 | 0.6  | 1.2                         |
| Female Adults              | 20 and up         | 0.1                            | 0.3                         | 25.0                          | 548 | 0.4  | 0.9                         |

**Table 3.2.3-1 Summary of the Estimated Daily Intake of Chondroitin Sulfate from Proposed Food-Uses of Peptan II in the U.S. by Population Group (2011-2012 NHANES Data)**

| Population Group | Age Group (Years) | All-Person Consumption (g/day) |                             | All-Users Consumption (g/day) |       |      |                             |
|------------------|-------------------|--------------------------------|-----------------------------|-------------------------------|-------|------|-----------------------------|
|                  |                   | Mean                           | 90 <sup>th</sup> Percentile | % Users                       | n     | Mean | 90 <sup>th</sup> Percentile |
| Male Adults      | 20 and up         | 0.2                            | 0.6                         | 28.1                          | 539   | 0.6  | 1.2                         |
| Total Population | All Ages          | 0.2                            | 0.5                         | 32.7                          | 2,683 | 0.5  | 1.0                         |

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

On a body weight basis, the total population mean and 90<sup>th</sup> percentile all-user intakes of chondroitin sulfate were determined to be 9.1 and 20.1 mg/kg body weight/day, respectively. Among the individual population groups, infants and young children were identified as having the highest mean and 90<sup>th</sup> percentile all-user intakes of any population group, of 25.0 and 55.3 mg/kg body weight/day, respectively (Table 3.2.3-2).

**Table 3.2.3-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Chondroitin Sulfate from Proposed Food-Uses of Peptan II in the U.S. by Population Group (2011-2012 NHANES Data)**

| Population Group           | Age Group (Years) | All-Person Consumption (mg/kg bw/day) |                             | All-Users Consumption (mg/kg bw/day) |       |      |                             |
|----------------------------|-------------------|---------------------------------------|-----------------------------|--------------------------------------|-------|------|-----------------------------|
|                            |                   | Mean                                  | 90 <sup>th</sup> Percentile | %                                    | n     | Mean | 90 <sup>th</sup> Percentile |
| Infants and Young Children | 0 to 2            | 8.8                                   | 29.0                        | 34.9                                 | 219   | 25.0 | 55.3                        |
| Children                   | 3 to 11           | 8.7                                   | 22.7                        | 58.6                                 | 890   | 14.8 | 28.5                        |
| Female Teenagers           | 12 to 19          | 3.8                                   | 11.8                        | 44.6                                 | 245   | 8.4  | 17.4                        |
| Male Teenagers             | 12 to 19          | 4.1                                   | 13.7                        | 44.8                                 | 235   | 9.3  | 21.5                        |
| Female Adults              | 20 and up         | 1.3                                   | 4.3                         | 24.9                                 | 540   | 5.2  | 11.6                        |
| Male Adults                | 20 and up         | 1.9                                   | 6.3                         | 28.2                                 | 533   | 6.8  | 14.5                        |
| Total Population           | All Ages          | 3.0                                   | 9.6                         | 32.7                                 | 2,662 | 9.1  | 20.1                        |

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

### 3.2.4 Summary of the Probable Consumption

In summary, on an all-user basis, the resulting mean and 90<sup>th</sup> percentile intakes of Peptan II by the total U.S. population from all proposed food-uses in the U.S., were estimated to be 1.8 g/person/day (36.5 mg/kg body weight/day) and 4.1 g/person/day (80.2 mg/kg body weight/day), respectively. Among the individual population groups, the highest mean intake of Peptan II was determined to be 2.3 g/person/day, as identified among both male teenagers and male adults (equivalent to 37.0 and 27.2 mg/kg body weight/day, respectively). Male teenagers were determined to have the highest 90<sup>th</sup> percentile intake of Peptan II at 4.9 g/person/day (85.9 mg/kg body weight/day). When intakes were expressed on a body weight basis, infants

and young children had the highest mean and 90<sup>th</sup> percentile all-user intakes of 100.0 and 221.0 mg/kg body weight/day, respectively. Although foods containing Peptan II are intended for consumption by adults only, infants, children, and teenagers are included in the intake estimate due to the possibility of consumption of foods containing Peptan II.

The estimated intakes of Peptan II were utilized to determine the subsequent intakes of the 2 primary components of this ingredient [*i.e.*, type II collagen ( $\leq 73\%$ ) and chondroitin sulfate (10 to 25%)]. In summary, among the total population, the mean and 90<sup>th</sup> percentile all-user intakes of type II collagen were determined to be 1.3 and 3.0 g/person/day, respectively (corresponding to 26.6 and 58.5 mg/kg body weight/day, respectively). For chondroitin sulfate, the total population mean and 90<sup>th</sup> percentile all-user intakes were determined to be 0.5 and 1.0 g/person/day, respectively (equivalent to 9.1 and 20.1 mg/kg body weight/day, respectively). Infants and young children had the highest intakes on a per body weight basis, and the resulting mean and 90<sup>th</sup> percentile intakes of these 2 components were 73.0 and 161.3 mg/kg body weight day, respectively for type II collagen; and 25.0 and 55.3 mg/kg body weight/day, respectively for chondroitin sulfate.

There were a number of assumptions included in the assessment, which mean that the resulting exposure estimates may be considered the 'worst case' values. For example, it has been assumed in both exposure assessments that all food products within a food category contain Peptan II at the maximum specified level of use. In reality, the levels added to specific foods will vary and it is unlikely that Peptan II will have 100% market penetration in all identified food categories. In addition, it is well established that the length of a dietary survey affects the estimated consumption of individual users. Short-term surveys, such as the typical 2- or 3-day dietary surveys, may overestimate the consumption of food products that are consumed relatively infrequently (Anderson, 1988).

#### **Part 4. §170.240 Self-Limiting Levels of Use**

At high concentrations, Peptan II may impart off-flavors to the foods or beverages to which it is added.

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

Peptan II has not been marketed prior to 1958, and the statutory basis for the conclusion that the use of Peptan II is GRAS is through scientific procedure, not through experience based on common use in food. It is worth mentioning though that the main constituents of Peptan II (*i.e.*, type II collagen and chondroitin sulfate) occur endogenously in humans, being components of connective tissues. Collagen (of various molecular types) is widely consumed from naturally occurring dietary sources. The FDA had no questions in response to a GRAS notification for

chondroitin sodium sulfate for use as an ingredient in foods at levels providing up to 1,200 mg/day (GRN 666) (U.S. FDA, 2017). Chondroitin sulfate also has a long history of consumption through dietary supplements in the U.S., Canada, and the EU. Therefore, the safety of Peptan II can be supported by the fact that its main constituents have been safely consumed in the diet (discussed further in Part 6).

## **Part 6. §170.250 Narrative**

The conclusion that Peptan II, as described herein, is GRAS under the conditions of its intended use in specified conventional food and beverage products is based on scientific procedures using generally available data and information pertaining to similar cartilage-derived products, collagen peptides, and chondroitin sulfate. This includes data and information related to the metabolic fate of these materials (described in Part 6.1 below), as well as the preclinical and clinical studies evaluating their safety (described in Part 6.2 below). A discussion of the findings of an independent Expert Panel specially convened by Rousselot to evaluate the GRAS status of Peptan II are discussed in Part 6.3 and a conclusion on GRAS status is presented in Part 6.4.

### **6.1 Absorption, Distribution, Metabolism, and Elimination**

As Peptan II is a product containing primarily 2 different, unbound components with different structures/chemistry, the pharmacokinetics of Peptan II will be related to that of its individual components (*i.e.*, chondroitin sulfate and type II collagen). Abundant published studies on the pharmacokinetics of these individual components were identified, the results of which are summarized below.

#### **6.1.1 Collagen**

Collagen, of various molecular types, is the primary protein component of connective tissues, and comprises approximately 30% of total body protein (Matsuda *et al.*, 2006; Zague, 2008). The collagen content of various animal proteins (*i.e.*, beef, beef liver, skinless chicken, lamb, sardines, and beef sausage) ranges from approximately 10 to 45% (Zarkadas, 1992; EI, 1995). Hydrolyzed collagen comprises water-soluble peptides with an average molecular weight of 2,000 to 6,000 Da, including the amino acids glycine (comprising approximately 35% of collagen peptides), L-proline and L-hydroxyproline (21%), alanine (11%), and L-hydroxylysine (PDRNS, 2008; Walrand *et al.*, 2008; Zague, 2008).

Mammals produce at least 30 different types of collagen, each occurring in a different tissue type (Lodish *et al.*, 2000; Nelson and Cox, 2000). Each type differs slightly in amino acid sequence and function; this is achieved through substitution of approximately a third of the component amino acids, while maintaining approximate contents of 35% glycine and 21% proline/hydroxyproline (Lodish *et al.*, 2000).

Collagen hydrolysates are enzymatically degraded in the gastrointestinal tract to small peptides and amino acid components (Walrand *et al.*, 2008). The uptake by enterocytes of small peptides and amino acids appears to occur *via* independent processes, with luminal and cytoplasmic peptidases limiting intact peptide absorption (Friedrich, 1982; Gardner, 1983; Webb, 1990). The amounts and types of peptides that are taken up intact are dependent on their ability to resist hydrolysis (Gardner, 1983). Peptide length, proline and hydroxyproline content, and heat damage are among the determinants of intact peptide *versus* individual amino acid absorption (Gardner, 1983; Roberts *et al.*, 1999). Although the hydrolysis of collagen may be slower due to its high content of L-proline and L-hydroxyproline, which form bonds with other amino acids that are highly resistant to enzymatic hydrolysis (PDRNS, 2008), the results of studies in guinea pigs, rats, and humans indicate that small peptides are absorbed faster from the small intestine than individual amino acids (Adibi and Morse, 1971, 1977; Gardner, 1982, 1983; Webb, 1990). Thus, the small peptides and individual amino acids derived from the type II collagen present in Peptan II are expected to be systemically bioavailable following ingestion.

The results of a study in which rats were administered single intragastric doses of gelatin (4,000 mg/kg body weight) or hydroxyproline (400 mg/kg body weight) provide further support that the digestion, absorption, and metabolism of hydrolyzed collagen are similar to that of other dietary proteins and peptides (Wang *et al.*, 2015). The results of studies in mice indicate that approximately 90% of an oral dose of radiolabeled hydrolyzed gelatin is absorbed from the gastrointestinal tract (as peptides ranging from 800 to 10,000 Da) within 3 to 6 hours of dosing (Zague, 2008; Watanabe-Kamiyama *et al.*, 2010). Hydrolyzed collagen is widely distributed, and has been detected in the skin, cartilage, liver, kidney, spleen, and skeletal muscle (Zague, 2008). The results of human studies indicate also that peptides and individual amino acids (including glycine, L-proline, L-hydroxyproline, and L-hydroxylysine) derived from various types of collagen hydrolysate are systemically absorbed following oral exposure (Iwai *et al.*, 2005; Ohara *et al.*, 2007; Walrand *et al.*, 2008).

### **6.1.2 Chondroitin Sulfate**

Although the reported percent bioavailability of chondroitin sulfate is highly variable (with higher bioavailability reported upon repeated compared to acute oral dosing), the results of studies in rats and dogs indicate significant oral absorption (up to 70% of the administered dose) of chondroitin sulfate after oral exposure, followed by distribution to the liver, intestines, kidney, cartilage, and synovial fluid (Palmieri *et al.*, 1990; Conte *et al.*, 1995; Adebowale *et al.*, 2002; Sakai *et al.*, 2002; Lamari *et al.*, 2006). The results of *ex vivo* studies indicate that orally administered chondroitin sulfate is degraded in the distal intestinal tract (followed by absorption of small chondroitin sulfate-derived oligosaccharides), with small amounts of intact compound absorbed in the proximal intestinal tract (Barthe *et al.*, 2004). In humans, 5 to 15% of a single oral dose of chondroitin sulfate has been reported to be absorbed (intact and as lower-

molecular weight oligosaccharides formed by digestive hydrolysis), with peak plasma concentrations reported 3 to 5 hours after dosing (Conte *et al.*, 1991, 1995; Ronca and Conte, 1993; Miraglia *et al.*, 2016).

## 6.2 Safety Studies

The intended uses of Rousselot's Peptan II are safe, suitable, and GRAS, based on a critical evaluation of published information characterizing the metabolism and safety of similar cartilage-derived products and their individual primary components (*i.e.*, various types of collagen and chondroitin sulfate), and their consumption as components of products similar to Peptan II. Support for this conclusion is discussed herein. As mentioned above, peptones, gelatin, and collagen are permitted for use as food ingredients in the U.S. In addition, commercial products derived from connective tissue and consisting of various types of collagen and/or chondroitin sulfate are currently marketed in the U.S. as dietary supplements and/or food ingredients.

### 6.2.1 Safety Studies on Products Similar to Peptan II

#### 6.2.1.1 Pre-Clinical

No animal studies have been conducted in which the toxicity of Peptan II was evaluated. However, the safety of the proposed use of Peptan II is supported by a lack of adverse effects reported in 2 animal studies of a product containing 10% hyaluronic acid, 60% type II collagen, and 20% chondroitin sulfate (BioCell® Collagen II) (Schauss *et al.*, 2007).

Schauss *et al.* (2007) conducted acute and 90-day toxicity studies on a product containing 10% hyaluronic acid, 60% type II collagen, and 20% chondroitin sulfate (BioCell® Collagen II). In the acute study, 6-week-old Sprague-Dawley rats (5/sex) were administered 5,000 mg BioCell® Collagen II/kg body weight in a fasting state, by gavage. This dose provided approximately 3,000 mg type II collagen and 1,000 mg chondroitin sulfate/kg body weight. Animals were observed for 14 days after dosing, and subjected to macroscopic tissue evaluation upon necropsy. No adverse effects were reported with respect to body weight, behavior, or gross pathology, and the median lethal dose (LD<sub>50</sub>) was determined to be >5,000 mg BioCell® Collagen II/kg body weight.

In the 90-day study, conducted in accordance with good laboratory practice and Organisation for Economic Cooperation and Development guidelines (OECD Guideline No. 408), 8-week-old Sprague-Dawley rats (10/sex/group) were housed individually and administered by gavage 0, 30, 300, or 1,000 mg BioCell® Collagen II/kg body weight/day in a fixed volume (100 mL) of

water<sup>8</sup>. Doses and required concentrations of test compound were calculated daily based on weekly-evaluated individual animal body weight. Food consumption was evaluated weekly, and animals were observed daily for mortality, activity, behavior, and clinical signs. Hematology and clinical biochemistry were evaluated from fasting blood samples collected within the final week of the study from all survivors. Upon necropsy, all animals were subject to gross and microscopic organ and tissue evaluation. No compound-related mortalities, adverse effects, or clinical signs were reported. The results of hematology, clinical chemistry, and gross and microscopic organ and tissue evaluations indicated no adverse compound-related effects, and a no-observed-adverse-effect level (NOAEL) of 1,000 mg BioCell® Collagen II/kg body weight/day, the highest dose tested, was determined. This dose is approximately equivalent to doses of 600 mg type II collagen and 200 mg chondroitin sulfate/kg body weight/day.

#### 6.2.1.2 Clinical

No clinical studies have been conducted in which the safety or efficacy of Peptan II was evaluated. However, the safety of Peptan II is supported by a lack of compound-related adverse effects reported in 10 human studies in which subjects consumed cartilage-derived products similar to Peptan II (Llopis-Miró *et al.*, 2012; Schauss *et al.*, 2012; Schwartz and Park, 2012; Di Cerbo *et al.*, 2015; Kanzaki *et al.*, 2015; Kumar *et al.*, 2015; Lopez *et al.*, 2015; Lugo *et al.*, 2016). In these 10 studies, healthy men and women (21 to 75 years of age) or those with osteoarthritis (n=527 with varying degrees of osteoarthritis) were provided with products based upon animal cartilage extracts containing various types of collagen, chondroitin sulfate, and/or other compounds believed to affect skin or joint physiology. The products (derived from bone, skin, or connective tissue obtained from sharks, fish, chicken, pigs, or cows) were consumed for 28 to 180 days, at doses providing up to 10 g collagen peptides and 600 mg chondroitin sulfate.

Six of the 10 identified human studies of products similar to Peptan II were conducted primarily to evaluate the efficacy of the products in the improvement of joint health, muscle recovery, and skin health (Llopis-Miró *et al.*, 2012; Schauss *et al.*, 2012; Schwartz and Park, 2012; Di Cerbo *et al.*, 2015; Lopez *et al.*, 2015). Although detailed safety evaluations were not included in these studies, adverse effects were monitored, and the study authors concluded in all studies that the intervention products were well tolerated, with no significant difference in the incidence, severity, or type of adverse effects reported between the placebo and intervention groups.

In the remaining 4 studies (in 3 publications), detailed evaluations of objective safety parameters were conducted, including vital signs, hematology, clinical chemistry, urinalysis, and adverse effects (Kanzaki *et al.*, 2015; Kumar *et al.*, 2015; Lugo *et al.*, 2016). In these 4 studies,

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<sup>8</sup> The administration protocol is described by Schauss *et al.* (2007) as: "The individual doses of the test product were determined based on each animal body weight and prepared within 2 h of administration as a 0.6% (low), 6.0% (intermediate), and 20% (high) w/v solution in distilled water. All doses were volumetrically equal (100 mL) and administered orally using a stainless steel ball-tipped gavage needle attached to a syringe."

a total of 351 adult men and women (40 to 75 years of age) with knee pain or knee osteoarthritis were assigned in a randomized, double-blind, placebo-controlled manner to consume products providing collagen peptides at doses of 1.2 mg/day (Lugo *et al.*, 2016), 45 mg/day (Kanzaki *et al.*, 2015), or 10 g/day (derived from porcine skin or bovine bone; Kumar *et al.*, 2015) and/or chondroitin sulfate at doses of 60 mg/day (Kanzaki *et al.*, 2015) or 1,200 mg/day (Lugo *et al.*, 2016) for 91 to 180 days. In 2 studies, the collagen and chondroitin components of these products are described specifically as type II collagen and chondroitin sulfate (Kanzaki *et al.*, 2015; Lugo *et al.*, 2016). In the remaining 2 studies, the products are described only as collagen peptides (derived from porcine skin or bovine bone) (Kumar *et al.*, 2015). Any statistically significant changes reported in these parameters remained within normal ranges, and were not considered physiologically relevant. Adverse effects also were monitored in these 4 studies, with none attributable to the study products.

In all 10 identified clinical studies of products similar to Peptan II, tolerance was reported to be good, and the study authors reported no safety concerns with respect to the consumption of the study product. Together, the results of these studies provide supportive evidence for the safety of Peptan II under the proposed conditions of use.

## **6.2.2 Studies on Components of Peptan II**

The primary components of Peptan II are common dietary constituents, and their safety has been evaluated extensively in the published literature. An overview of the safety of collagen and chondroitin sulfate is provided herein.

### *6.2.2.1 Collagen*

As mentioned above, the types of collagen proteins differ slightly in their amino acid sequence and function due to substitution of approximately a third of the component amino acids, while maintaining approximate contents of 35% glycine and 21% proline/hydroxyproline (Lodish *et al.*, 2000). Since the various types of collagen are expected to be digested and utilized according to typical pathways for dietary proteins, there is no expected difference between the various types of collagen with respect to their safety.

In 2005, the Institute of Medicine (IOM) evaluated data on protein and individual amino acids to determine dietary reference values for these nutrients (IOM, 2005). A Recommended Dietary Allowance<sup>9</sup> (RDA) of 0.8 g/kg body weight was set for protein (good quality) for adult males and females. The IOM concluded that there were insufficient data to set a Tolerable Upper Intake Level for total protein or for any of the amino acids (IOM, 2005). In addition, the IOM noted that “the risk of adverse effects resulting from excess intakes of protein from foods appears to be

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<sup>9</sup> The average daily dietary nutrient intake level sufficient to meet the nutrient requirement of nearly all (97 to 98%) healthy individuals in a particular life stage and gender group (IOM, 2005).

very low at the highest estimated intake[s]” (IOM, 2005). The IOM also estimated background dietary protein intakes for the U.S. population using the Continuing Survey of Food Intakes by Individuals (CSFII, 1994-1996, 1998) (IOM, 2005). The mean adult intake for protein ranged from approximately 56 g (adult females over the age of 70) to 104 g (19- to 30-year-old males)/day (IOM, 2005). At the 90<sup>th</sup> percentile, adult protein intakes ranged from 76 g/day for adult females over 70 years of age to 142 g/day for 19- to 30-year-old males. In infants and children, mean and 90<sup>th</sup> percentile intakes ranged from 15.9 to 62.5, and 21.9 to 81.9 g/day, respectively. The mean and 90<sup>th</sup> percentile total population intakes were estimated to be approximately 75 and 114 g protein/day, respectively. Using the 90<sup>th</sup> percentile total population intake of protein (114 g/day) and a maximum protein collagen content (45%; assuming all of the dietary protein is derived from animal sources), daily collagen consumption may be estimated to be up to 51.3 g.

JECFA (1971), SCOGS (FASEB, 1975), and the Cosmetic Ingredient Review (CIR, 1985) reviewed the safety of oral and dermal exposure to gelatin (*i.e.*, hydrolyzed collagen) and concluded that they had no safety concerns with respect to the use of the compound as a food or cosmetic ingredient. According to the SCOGS (FASEB, 1975), gelatin is an ingredient in commonly consumed foods, and has been used as such for over 165 years. Although it is considered to be of low nutritional value, the SCOGS noted that there was no documented evidence of adverse effects attributable to gelatin (FASEB, 1975). Gelatin also is used as an excipient in many oral and parenteral pharmaceutical formulations (FASEB, 1975; Rowe *et al.*, 2009). Allergic responses have been reported rarely, and are primarily associated with parenteral exposure to gelatin (FASEB, 1975; Rowe *et al.*, 2009). The SCOGS (FASEB, 1975) concluded that “There is no evidence in the available information on gelatin that demonstrates or suggests reasonable grounds to suspect a hazard to the public when it is used at levels that are now current or that might reasonably be expected in the future”.

#### 6.2.2.1.1 Pre-Clinical

The results of several recently published studies also provide supportive evidence of safety for the intake of type II collagen under the proposed uses of Peptan II as a food ingredient. Increased absolute kidney weight (attributed to very high dietary protein intakes) was reported compared to control animals in a study in which juvenile rats were given diets providing 16,600 mg porcine-derived collagen (1,000 to 30,000 Da; molecular type not reported)/kg body weight/day for 4 weeks (Wu *et al.*, 2004), and in ovariectomized spontaneously hypertensive stroke-prone (SHSRP) rats given drinking water providing approximately 10,000 mg low-molecular weight collagen (predominantly type I)/kg body weight/day for 20 weeks (Watanabe-Kamiyama *et al.*, 2010)<sup>10</sup>. No additional compound-related effects were reported in either study.

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<sup>10</sup> Relative kidney weights were not reported in either study, nor were histopathological examinations of the kidneys conducted.

Wu *et al.* (2004) described the observed increase in kidney weight in animals consuming 16,600 mg collagen/kg body weight/day for 4 weeks as a hypertrophic response to extremely high dietary protein intake, and noted that this level of intake (*i.e.*, approximately 996 g collagen/day for a 60-kg human) would be highly unlikely in human consumers of products containing collagen. Watanabe-Kamiyama *et al.* (2010) also attributed the reported increase in kidney weight to very high dietary protein intake, with the administered dose of 10,000 mg collagen/kg body weight/day equivalent to approximately 600 g collagen/day for a 60-kg human. In neither of these 2 studies was the observed increase in kidney weight attributed to a toxicological effect of collagen peptides specifically; rather the increase in kidney weight was attributed to extremely high dietary protein intakes (Wu *et al.*, 2004; Watanabe-Kamiyama *et al.*, 2010).

No adverse compound-related effects were reported in several other studies in which mice or piglets were given 200 mg collagen peptides/kg body weight/day, or rats were given 1,660 mg/kg body weight/day, for 6 to 9 weeks (Wu *et al.*, 2004; Matsuda *et al.*, 2006; Tanaka *et al.*, 2009).

#### 6.2.2.1.2 Clinical

No compound-related adverse effects were reported in a series of studies in healthy adults, post-menopausal women, adults with joint pain, or adults with osteoarthritis, type 2 diabetes, mild hypertension, or pressure ulcers, who consumed products composed primarily of isolated hydrolyzed collagen of various molecular types (rather than extracts of animal tissues comprising multiple components, as described in Part 6.2.1.2 above), providing up to 40 g hydrolyzed collagen/day (alone or with low doses of other cartilage-derived compounds including hyaluronic acid or chondroitin sulfate) for up to 20 months (Barnett *et al.*, 1998; Lee *et al.*, 2006; Clark *et al.*, 2008; Benito-Ruiz *et al.*, 2009; Cúneo *et al.*, 2010; Zhu *et al.*, 2010; Trč and Bohmová, 2011; Bruyère *et al.*, 2012; Llopis-Miró *et al.*, 2012; Schauss *et al.*, 2012; Schwartz and Park, 2012; Kouguchi *et al.*, 2013; Jiang *et al.*, 2014; Wang *et al.*, 2015; Inoue *et al.*, 2016; Lugo *et al.*, 2016). These studies, and the endogenous nature of collagen peptides, support the safety of type II collagen intake from the proposed uses of Peptan II (providing up to 3.0 g type II collagen/day).

#### 6.2.2.2 Chondroitin Sulfate

##### 6.2.2.2.1 Pre-Clinical

In a recently published animal study of chondroitin sulfate-sodium, an NOAEL of 1,000 mg/kg body weight/day was reported based on a lack of compound-related adverse effects on mortality, clinical signs, body weight, food consumption, hematology, clinical chemistry, urinalysis, organ weights, histopathology, or gross necropsy in Sprague-Dawley rats (10/sex/group) administered chondroitin sulfate-sodium by gavage at doses of 0, 250, 500, or

1,000 mg/kg body weight/day for 90 days (Miraglia *et al.*, 2016). The results of a bacterial reverse mutation assay<sup>11</sup>, a chromosomal aberration assay<sup>12</sup>, and an *in vitro* mammalian cell gene mutation assay<sup>13</sup> also were reported in this publication to be negative.

#### 6.2.2.2.2 Clinical

Hathcock and Shao (2007) reviewed 7 clinical studies and 4 meta-analyses in a risk assessment of dietary supplementation with chondroitin sulfate. The only adverse event identified among the studies reviewed (encompassing  $\geq 2,312$  subjects) was a single case of gastritis in a study in which 165 subjects consumed 1,200 mg chondroitin sulfate/day (or placebo) for 3 years (Verbruggen *et al.*, 2002). Hathcock and Shao (2007) noted that this adverse event was not likely compound-related, and concluded that 1,200 mg/day (the highest dose identified) is the observed safe level (OSL) for chondroitin sulfate. Hathcock and Shao (2007) noted also that this dose is not a true NOAEL, and that the true lowest-observed-adverse-effect level is likely to be much higher. Hathcock and Shao (2007) noted that the available human data pertaining to the safety of chondroitin sulfate were sufficient to establish an OSL, and that animal data did not need to be evaluated.

In a recently published Cochrane Review (Singh *et al.*, 2015) on the efficacy and safety of chondroitin in subjects with osteoarthritis, the authors reported conducting a meta-analysis including 43 randomized, controlled intervention studies in which a total of 9,110 adult men and women ( $\geq 18$  years of age) with varying degrees of osteoarthritis consumed chondroitin or chondroitin sulfate (at doses of 200 to 2,000 mg/day), alone or in combination with glucosamine (or placebo). In three of the studies included in this meta-analysis, doses  $> 1,200$  mg chondroitin sulfate/day were consumed (Mazières *et al.*, 1992; Debi *et al.*, 2000; Nasonova *et al.*, 2001). As neither of these studies was published in English, and one study (Debi *et al.*, 2000) involved intravenous administration of chondroitin sulfate, their relevance to the safety of the use of Peptan II as a food ingredient could not be determined. Considering all 43 studies, Singh *et al.* (2015) concluded that “chondroitin did not result in statistically significant numbers of adverse events or withdrawals due to adverse events” compared to placebo or active control products.

Ten additional human studies not included in the meta-analyses conducted by Hathcock and Shao (2007) and Singh *et al.* (2015) were identified in the literature. In these studies, a total of 4,343 adults with osteoarthritis or chronic knee pain consumed up to 1,200 mg chondroitin sulfate/day (with or without glucosamine sulfate or hydrochloride) for 3 to 24 months (Mazières

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<sup>11</sup> Test conducted using *S. typhimurium* (TA1535, TA1537, TA98, and TA100) and *E. coli* (WP2uvrA), with up to 5,000  $\mu\text{g}$  chondroitin sulfate sodium/plate, with or without metabolic activation.

<sup>12</sup> Test conducted *in vitro* using Chinese hamster ovary cells, with chondroitin sulfate sodium at concentrations of  $\leq 5,000$   $\mu\text{g}/\text{mL}$ , with or without metabolic activation.

<sup>13</sup> Test conducted using mouse lymphoma L5178Y cells, with chondroitin sulfate sodium at concentrations of  $\leq 5,000$   $\mu\text{g}/\text{mL}$ , with or without metabolic activation.

*et al.*, 2007; Zegels *et al.*, 2013; Hochberg *et al.*, 2014, 2016; Fransen *et al.*, 2015; Provenza, *et al.*, 2015; Herrero-Beaumont *et al.*, 2016; Lugo *et al.*, 2016; Roman-Blas *et al.*, 2016). In a single-dose study, 24 healthy adult subjects were given 2,400 mg chondroitin sulfate (microbial- or bovine-derived) in a fasting state (Miralgia *et al.*, 2016). Adverse events possibly related to treatment were reported rarely, and were reported with equal frequency in treatment and control groups, where applicable. The study authors concluded that chondroitin sulfate was well tolerated, and did not raise any safety concerns with respect to the long-term use of chondroitin sulfate in the management of osteoarthritis or knee pain. The results of these studies, in addition to the endogenous nature of chondroitin sulfate, further support that the intake of chondroitin sulfate from the proposed uses of Peptan II (providing up to 1.0 g chondroitin sulfate/day) would not pose any safety concerns. Although the data required to estimate the background dietary intakes of chondroitin sulfate were not available, typical supplementary doses of this component of up to 1,200 mg/day are commercially available and have been reported in human studies.

### **6.2.3 Allergenicity**

Although no studies designed specifically to investigate the allergenicity of Peptan II or its components were identified, compound-related allergic reactions were reported rarely in the identified authoritative reviews, assessments, meta-analyses, or clinical studies. Hochberg *et al.* (2016) reported 1 allergic reaction among 264 subjects given 1,200 mg chondroitin sulfate and 1,500 mg glucosamine hydrochloride/day for 6 months. Schauss *et al.* (2012) reported 1 mild allergic reaction (considered probably related to treatment) among 40 subjects consuming 2 g BioCell Collagen II/day for 12 weeks. In 2 reviews of the safety and efficacy of collagen, the authors noted that allergenic responses have been reported rarely, and are primarily associated with parenteral exposure to gelatin (FASEB, 1975; Rowe *et al.*, 2009).

As proteins with molecular weights less than 5,000 Da tend to be poorly allergenic (Kuby, 1997), the partially hydrolyzed proteins present in Peptan II are not expected to be allergenic. However, potential cases of hypersensitivity to porcine proteins may exist. Thus, Rousselot recommends that manufacturers of food products containing Peptan II ensure that this product is in compliance with the local regulation in force.

### **6.3 Summary**

The totality of information and the weight of the available evidence is sufficient to support a conclusion that the intended use of Peptan II at a level up to 5.0% in variety of food products intended for consumption by adults [including active nutrition and nutritionally complete bars, granola bars, enhanced fortified water beverages, sports nutrition gels, fortified flavored milk beverages (excluding milkshakes), enhanced or fortified fruit-flavored beverages, and gummies] (providing up to 1.8 g Peptan II/person/day, including up to 1.3 g type II collagen and 0.5 g

chondroitin sulfate/day) is safe and suitable and GRAS. Pivotal supporting data include the following:

- 1) Peptan II is manufactured from porcine trachea cartilage in accordance with a food safety system compliant with HACCP principles, using safe and suitable food grade raw materials, reagents, and processing aids. This process results in a final product that consistently meets specifications and is stable for  $\geq 12$  months under ambient storage conditions.
- 2) Peptan II is a product containing primarily 2 different, unbound components with different structures/chemistry (*i.e.*, chondroitin sulfate and type II collagen). Peptones, gelatin, and various molecular types of collagen are natural components of foods, and are permitted for use as food ingredients in the U.S. Commercial products similar to Peptan II (*i.e.*, derived from animal cartilage), or mixed products containing various molecular types of collagen and/or chondroitin sulfate, are currently marketed in the U.S. as dietary supplements and/or food ingredients. Chondroitin sulfate sodium is GRAS for use in foods at levels providing up to 1,200 mg/day (GRN 666) (U.S. FDA, 2017).
- 3) Collagen hydrolysates are enzymatically degraded in the gastrointestinal tract to small peptides and amino acid components (Walrand *et al.*, 2008). Peptides and individual amino acids derived from collagen are well absorbed from the gastrointestinal tract and widely distributed in rats, mice, and humans (Iwai *et al.*, 2005; Ohara *et al.*, 2007; Walrand *et al.*, 2008; Zague, 2008; Watanabe-Kamiyama *et al.*, 2010; Wang *et al.*, 2015). Chondroitin sulfate also is absorbed (5 to 15% of an oral dose in humans) as the parent compound and small oligosaccharides and widely distributed (Palmieri *et al.*, 1990; Conte *et al.*, 1991, 1995; Ronca and Conte, 1993; Adebowale *et al.*, 2002; Sakai *et al.*, 2002; Lamari *et al.*, 2006; Miraglia *et al.*, 2016).
- 4) No compound-related adverse effects were reported in rats in an acute or 90-day study of a product similar to Peptan II (containing 10% hyaluronic acid, 60% type II collagen, and 20% chondroitin sulfate) (Schauss *et al.*, 2007). A NOAEL of 1,000 mg/kg body weight/day, the highest dose tested, was reported in the 90-day study. Likewise, no compound-related adverse effects were reported in 10 human studies of cartilage-derived products similar to Peptan II at doses providing up to 10 g collagen peptides and 600 mg chondroitin sulfate/day for up to 180 days (Llopis-Miró *et al.*, 2012; Schauss *et al.*, 2012; Schwartz and Park, 2012; Di Cerbo *et al.*, 2015; Kanzaki *et al.*, 2015; Kumar *et al.*, 2015; Lopez *et al.*, 2015; Lugo *et al.*, 2016).
- 5) Collagen (various molecular types) is endogenous in humans and has a long history of use as an ingredient in foods and pharmaceuticals, with no documented compound-related adverse events, and is a natural component of the diet (JECFA, 1971; FASEB, 1975; CIR, 1985; Rowe *et al.*, 2009). No compound-related adverse effects were reported in mice, rats, or piglets at doses up to 1,660 mg collagen peptides/kg body weight/day (administered for

up to 9 weeks) (Wu *et al.*, 2004; Matsuda *et al.*, 2006; Tanaka *et al.*, 2009), or in humans at doses up to 40 g collagen peptides/day, consumed for up to 20 months (Barnett *et al.*, 1998; Lee *et al.*, 2006; Clark *et al.*, 2008; Benito-Ruiz *et al.*, 2009; Cúneo *et al.*, 2010; Zhu *et al.*, 2010; Trč and Bohmová, 2011; Bruyère *et al.*, 2012; Llopis-Miró *et al.*, 2012; Schauss *et al.*, 2012; Schwartz and Park, 2012; Kouguchi *et al.*, 2013; Jiang *et al.*, 2014; Wang *et al.*, 2015; Inoue *et al.*, 2016; Lugo *et al.*, 2016).

- 6) Chondroitin sulfate is endogenous in humans and has a long history of use in dietary supplement products in the U.S., Canada, and the EU. A NOAEL of 1,000 mg chondroitin sulfate-sodium/kg body weight/day (the highest dose tested) was determined based on a lack of adverse effects reported in a 90-day study in rats (Miraglia *et al.*, 2016). The results of 3 *in vitro* genotoxicity studies demonstrate that chondroitin sulfate lacks genotoxic potential (Miraglia *et al.*, 2016). An OSL of 1,200 mg chondroitin sulfate/day was determined by Hathcock and Shao (2007) based on a lack of adverse effects in a number of human studies. No compound-related adverse effects were reported in a Cochrane review of chondroitin sulfate or in 10 additional human studies identified in the literature, at doses up to 1,200 mg/day, consumed for up to 24 months (Mazières *et al.*, 2007; Zegels *et al.*, 2013; Hochberg *et al.*, 2014, 2016; Fransen *et al.*, 2015; Provenza, *et al.*, 2015; Singh *et al.*, 2015; Herrero-Beaumont *et al.*, 2016; Lugo *et al.*, 2016; Roman-Blas *et al.*, 2016).
- 7) Compound-related allergic reactions were reported rarely among the identified authoritative reviews, assessments, meta-analyses, or clinical studies of products similar to Peptan II, collagen (various molecular types), or chondroitin sulfate. As proteins with molecular weights less than 5,000 Da tend to be poorly allergenic (Kuby, 1997), the partially hydrolyzed proteins present in Peptan II are not expected to be allergenic.

## 6.4 Expert Panel Evaluation

A Panel of Experts (the Expert Panel), who are qualified by scientific training and experience to evaluate the safety of food ingredients, has unanimously concluded on the GRAS status of Peptan II under the conditions of its intended use. The Panel consisted of the following qualified scientific experts: Professor Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University School of Medicine); Professor Robert J. Nicolosi, Ph.D. (University of Massachusetts Lowell); and Professor John A. Thomas, Ph.D. (Indiana University School of Medicine).

The Expert Panel, independently and collectively, critically evaluated the data and information summarized herein and concluded that the intended uses of Peptan II in a variety of food products intended for consumption by adults [including active nutrition and nutritionally complete bars, granola bars, enhanced fortified water beverages, sports nutrition gels, fortified flavored milk beverages (excluding milkshakes), enhanced or fortified fruit-flavored beverages, and gummies], meeting appropriate food-grade specifications and manufactured according to a food safety system compliant with HACCP principles, are safe, suitable, and GRAS based on

scientific procedures. Furthermore, it is the Expert Panel's opinion that other qualified and competent scientists reviewing the same publicly available information would reach the same conclusion. 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 Peptan II is presented in Appendix A.

## **6.5 Conclusion**

Based on the above data and information presented herein, Rousselot has concluded that the intended uses of Peptan II in specified conventional food and beverage products, as described in Part 1.3, are GRAS based on scientific procedures. The GRAS status of Peptan II is further supported by the unanimous consensus rendered by an independent Panel of Experts, qualified by experience and scientific training to evaluate the safety of food ingredients, who concluded that the intended use of Peptan II in conventional food and beverage products, as described herein, is GRAS.

Peptan II therefore may be marketed and sold for its intended purpose in the U.S. without the promulgation of a food additive regulation under Title 21, Section 170.3 of the Code of Federal Regulations.

## Part 7. §170.255 List of Supporting Data and Information

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| 184—Direct food substances affirmed as generally recognized as safe     | 184.1027  | Mixed carbohydrase and protease enzyme product                                     |
|   | 184.1366  | Hydrogen peroxide  |
|   | 184.1420  | Lipase enzyme preparation derived from <i>Rhizopus niveus</i> [Diatomaceous earth] |
|   | 184.1553  | Peptones   |
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## **Appendix A**

### **Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of Hydrolyzed Porcine Cartilage (Peptan II) for Use as a Food Ingredient in the United States**

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# **Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of Hydrolyzed Porcine Cartilage (Peptan II) for Use as a Food Ingredient in the United States**

**February 23, 2017**

At the request of Rousselot BVBA (Rousselot), Intertek Scientific and Regulatory Consultancy convened an Expert Panel of independent scientists, qualified by their scientific training and relevant national and international experience to evaluate the safety of food ingredients, to conduct a critical and comprehensive evaluation of the available pertinent data and information on hydrolyzed porcine cartilage (Peptan II), and to determine whether the proposed use of hydrolyzed porcine cartilage (Peptan II) at levels up to 4.0% in a variety of food products [including active nutrition and nutritionally complete bars, granola bars, enhanced fortified water beverages, sports nutrition gels, fortified flavored milk beverages (excluding milkshakes), enhanced or fortified fruit-flavored beverages, and fortified gummies], providing up to 1.8 g/person/day, is Generally Recognized as Safe (GRAS), based on scientific procedures. The Expert Panel consisted of the below-signed qualified scientific experts: Professor Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University School of Medicine); Professor Robert J. Nicolosi, Ph.D. (University of Massachusetts Lowell); and Professor John A. Thomas, Ph.D. (Indiana University School of Medicine). For purposes of the Expert Panel's evaluation, "safe" or "safety" indicates that there is a reasonable certainty of no harm under the intended conditions of use of the ingredient in foods, as stated in 21 CFR §170.3(i) (U.S. FDA, 2016).

The Expert Panel, independently and collectively critically examined a comprehensive package of scientific information and data pertinent to Peptan II (and its primary components, collagen and chondroitin sulfate) compiled from the literature and other published sources through November 25, 2016. This information was presented in a dossier, "Documentation Supporting the Evaluation of Hydrolyzed Porcine Cartilage (Peptan II) as Generally Recognized as Safe (GRAS) for Use as a Food Ingredient in the United States". In addition, the Expert Panel evaluated other information deemed appropriate, necessary, or pertinent, to the safety of the conditions of use of Peptan II and its primary components (type II collagen and chondroitin sulfate). The information evaluated by the Expert Panel included the method of manufacture, product specifications and analytical data, the conditions of intended use of Peptan II, dietary intake estimates for the proposed use, and a comprehensive assessment of the available scientific literature pertaining to the safety of Peptan II, similar cartilage-derived products, various types of collagen, and chondroitin sulfate.

Following independent and collaborative critical evaluation of such data and information, the Expert Panel met *via* teleconference on February 23, 2017. The Expert Panel unanimously concluded that Peptan II, meeting appropriate food-grade specifications and manufactured in accordance with

a Food Safety system compliant with hazard analysis and critical control points (HACCP) principles, is GRAS for use at a level of up to 4.0% in a variety of food products intended for consumption by adults [including active nutrition and nutritionally complete bars, granola bars, enhanced fortified water beverages, sports nutrition gels, fortified flavored milk beverages (excluding milkshakes), enhanced or fortified fruit-flavored beverages, and fortified gummies], providing up to 1.8 g/person/day. The GRAS determination was based on scientific procedures, and a summary of the basis for the Expert Panel's conclusion is provided below.

## **SUMMARY AND BASIS FOR GRAS DETERMINATION**

Peptan II is manufactured from porcine trachea cartilage in accordance with a Food Safety system compliant with HACCP principles, using safe and suitable food grade raw materials, reagents, and processing aids permitted for use in food in the United States (U.S.) and/or determined to be GRAS for use in the manufacture of Peptan II. The Expert Panel considered the adherence of Peptan II to Rousselot's established specifications to support its suitability, under its conditions of manufacture, for use as a food ingredient. Peptan II has a shelf-life of 12 months when stored under ambient storage conditions. The Expert Panel reviewed batch analyses from 3 lots of Peptan II, as provided by Rousselot, demonstrating that the ingredient is manufactured in a reproducible manner and is consistently compliant with physical, chemical, and microbiological specifications established by Rousselot.

Peptones (*i.e.*, mixtures of polypeptides, oligopeptides, and amino acids produced by hydrolysis or denaturation of proteins, including animal tissue and gelatin), gelatin (or hydrolyzed collagen of unspecified molecular types), hydrolyzed gelatin, and collagen (molecular type not specified) are natural components of foods, and are permitted for use as food ingredients in the U.S. (21 CFR §172.230, 21 CFR §184.1553 - U.S. FDA, 2016) (FASEB, 1975; Moskowitz, 2000). In addition, commercial products similar to Peptan II (*i.e.*, extracts of connective tissue consisting of collagen and/or chondroitin, including BioCell Collagen Type II, Summit Nutritionals Cartilage Powder, and Copalis Collagen Type II/Cartilage products) are currently marketed in the U.S. as dietary supplements and/or food ingredients. The U.S. Food and Drug Administration had no questions in response to a GRAS notification for pork collagen (molecular type not specified) when used as a binder or purge-reducing additive in meat or meat-type products at levels of 1 to 3.5% (U.S. FDA, 1999).

Chondroitin sulfate is used in dietary supplements worldwide, is considered an "old" dietary ingredient in the U.S., and is listed on the Council for Responsible Nutrition (CRN) list of dietary ingredients "grandfathered" under the *Dietary Supplement Health and Education Act of 1994* (DSHEA, 1994) and the United Natural Products Alliance (UNPA) (CRN, 1998; UNPA, 1999). In Canada and the European Union (EU), chondroitin sulfate is permitted for use in dietary

supplement products, with permitted doses of  $\leq 1,200$  mg/day (Health Canada, 2008; Boots UK Limited, 2016; Holland & Barrett Retail Limited, 2016; Vitabiotics Ltd., 2016)<sup>1</sup>.

Collagen, of various molecular types, is the primary protein component of connective tissues, and comprises approximately 30% of total body protein (Matsuda *et al.*, 2006; Zague, 2008). The collagen content of various animal proteins (*i.e.*, beef, beef liver, skinless chicken, lamb, sardines, and beef sausage) ranges from approximately 10 to 45% (Zarkadas, 1992; El, 1995). The Institute of Medicine (IOM) also estimated background dietary protein intakes for the U.S. population using the Continuing Survey of Food Intakes by Individuals (CSFII, 1994-1996, 1998) (IOM, 2005). The mean adult intake for protein ranged from approximately 56 g (adult females over the age of 70) to 104 g (19- to 30-year-old males)/day (IOM, 2005). At the 90<sup>th</sup> percentile, adult protein intakes ranged from 76 g/day for adult females over 70 years of age to 142 g/day for 19- to 30-year-old males. In infants and children, mean and 90<sup>th</sup> percentile intakes ranged from 15.9 to 62.5, and 21.9 to 81.9 g/day, respectively. The mean and 90<sup>th</sup> percentile total population intakes were estimated to be approximately 75 and 114 g protein/day, respectively. Using the 90<sup>th</sup> percentile total population intake of protein (114 g/day) and a maximum protein collagen content (45%; assuming all of the dietary protein is derived from animal sources), daily collagen consumption may be estimated to be 51.3 g.

Although the data required to estimate the background dietary intakes of chondroitin sulfate were not available, typical supplementary doses of this component of up to 1,200 mg/day are commercially available and have been reported in human studies.

The proposed uses of Peptan II in a variety of food products intended for consumption by adults [including active nutrition and nutritionally complete bars, granola bars, enhanced fortified water beverages, sports nutrition gels, fortified flavored milk beverages (excluding milkshakes), enhanced or fortified fruit-flavored beverages, and fortified gummies] were estimated to result in a mean intake of 1.8 g/person/day (36.5 mg/kg body weight/day) and a 90<sup>th</sup> percentile intake of 4.1 g/person/day (80.2 mg/kg body weight/day) in the total population. The individual population group with the highest estimated intakes resulting from the proposed food use was male teenagers, who displayed mean and 90<sup>th</sup> percentile intakes of 2.3 g/person/day (27.2 to 37.0 mg/kg body weight/day) and 4.9 g/person/day (85.9 mg/kg body weight/day), respectively.

The estimated intakes of Peptan II were utilized to determine the subsequent intakes of the 2 primary components of this ingredient [*i.e.*, type II collagen ( $\leq 73\%$ ) and chondroitin sulfate (10 to 25%)]. Among the total population, the mean and 90<sup>th</sup> percentile all-user intakes of type II collagen were determined to be 1.3 and 3.0 g/person/day, respectively (corresponding to 26.6 and 58.5 mg/kg body weight/day, respectively). For chondroitin sulfate, the total population mean and 90<sup>th</sup>

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<sup>1</sup> <http://www.boots.com/webapp/wcs/stores/servlet/EndecaSearchListerView?storeId=10052&langId=-1&catalogId=10551&stReq=1&searchTerm=chondroitin+sulphate&newDepSearch=&x=0&y=0#container>  
<http://www.vitabiotics.com/jointace/max>  
<http://www.hollandandbarrett.com/shop/product/holland-barrett-max-strength-glucosamine-chondroitin-caplets-60041631>

percentile all-user intakes were determined to be 0.5 and 1.0 g/person/day, respectively (equivalent to 9.1 and 20.1 mg/kg body weight/day, respectively).

The National Health and Nutrition Examination Survey is a short term survey (*i.e.*, 2 days of data per cycle), and can therefore overestimate long-term consumption patterns. Estimates derived from these data are therefore considered to represent a worst-case illustration of potential intakes. Additionally, the assessments generated based on the proposed use level for Peptan II are based on the assumption that all product categories consumed contain the maximum indicated quantity of Peptan II, which also may lead to an overestimation of the impact of the proposed use levels on the consumption of Peptan II among consumers of these products. Estimates of the intakes of type II collagen and chondroitin sulfate were calculated using the average type II collagen content of the 3 representative batches of Peptan II provided (*i.e.*, approximately 73%), and the maximum specified chondroitin sulfate content (*i.e.*, 25%).

Since Peptan II is a product containing primarily 2 different, unbound components with different structures/chemistry (*i.e.*, chondroitin sulfate and type II collagen), the Expert Panel reviewed published information characterizing the metabolic fate of chondroitin sulfate and type II collagen.

Hydrolyzed collagen comprises water-soluble peptides with an average molecular weight of 2,000 to 6,000 Da, including the amino acids glycine (comprising approximately 35% of collagen peptides), L-proline and L-hydroxyproline (21%), alanine (11%), and L-hydroxylysine (PDRNS, 2008; Walrand *et al.*, 2008; Zague, 2008). Collagen hydrolysates are enzymatically degraded in the gastrointestinal tract to small peptides and amino acid components (Walrand *et al.*, 2008). The results of studies in rats, mice, and humans demonstrate that peptides and individual amino acids derived from gelatin and hydrolyzed gelatin are well absorbed from the gastrointestinal tract and widely distributed (Iwai *et al.*, 2005; Ohara *et al.*, 2007; Walrand *et al.*, 2008; Zague, 2008; Watanabe-Kamiyama *et al.*, 2010; Wang *et al.*, 2015).

The results of studies in rats and dogs indicate significant absorption of chondroitin sulfate after oral exposure (up to 70% of the administered dose), followed by distribution to the liver, intestines, kidney, cartilage, and synovial fluid (Palmieri *et al.*, 1990; Conte *et al.*, 1995; Adebowale *et al.*, 2002; Sakai *et al.*, 2002; Lamari *et al.*, 2006). The results of *ex vivo* studies indicate that orally administered chondroitin sulfate is degraded in the distal intestinal tract (followed by absorption of small chondroitin sulfate-derived oligosaccharides), with small amounts of intact compound absorbed in the proximal intestinal tract (Barthe *et al.*, 2004). In humans, 5 to 15% of a single oral dose of chondroitin sulfate has been reported to be absorbed (as lower-molecular weight oligosaccharides formed by digestive hydrolysis), with peak plasma concentrations reported 3 to 5 hours after dosing (Conte *et al.*, 1991, 1995; Ronca and Conte, 1993; Miraglia *et al.*, 2016).

No animal or human studies were identified in which the safety or efficacy of Peptan II was evaluated. However, the safety of the proposed use of Peptan II is supported by a lack of adverse effects reported in 2 animal studies of a product containing 10% hyaluronic acid, 60% type II

collagen, and 20% chondroitin sulfate (BioCell® Collagen II) (Schauss *et al.*, 2007). In an acute toxicity study, no adverse effects with respect to body weight, behavior, or gross pathology were reported in Sprague-Dawley rats over 14 days following administration of 5,000 mg BioCell® Collagen II/kg body weight (providing approximately 3,000 mg type II collagen and 1,000 mg chondroitin sulfate/kg body weight), by gavage.

No compound-related adverse effects were reported with respect to hematology, clinical biochemistry, mortality, behavior, body weight, food consumption, clinical signs, or gross and microscopic tissue evaluation in a 90-day study in Sprague-Dawley rats orally administered by gavage the following doses in a fixed volume of water<sup>2</sup> (100 mL/animal): 0 (water vehicle), 30, 300, or 1,000 mg BioCell® Collagen II/kg body weight/day (up to approximately 600 mg type II collagen and 200 mg chondroitin sulfate/kg body weight/day). A no-observed-adverse-effect level (NOAEL) of 1,000 mg BioCell® Collagen II/kg body weight/day, the highest dose tested, was therefore determined (Schauss *et al.*, 2007).

The safety of Peptan II is further supported by a reported lack of compound-related adverse effects in 10 human studies in which subjects consumed cartilage-derived products similar to Peptan II (Llopis-Miró *et al.*, 2012; Schauss *et al.*, 2012; Schwartz and Park, 2012; Di Cerbo *et al.*, 2015; Kanzaki *et al.*, 2015; Kumar *et al.*, 2015; Lopez *et al.*, 2015; Lugo *et al.*, 2016). In these 10 studies, a total of 654 healthy men and women (21 to 75 years of age) or those with osteoarthritis (n=527 with varying degrees of osteoarthritis) were provided products based upon animal cartilage extracts (derived from connective tissue obtained from sharks, fish, chicken, pigs, or cows) containing collagen, chondroitin, and/or other compounds believed to affect skin or joint physiology. The products were consumed for up to 180 days, at daily doses providing up to 10 g collagen peptides and 600 mg chondroitin sulfate.

Given the general lack of safety concerns regarding cartilage-based products similar to Peptan II, many studies on these products were conducted primarily to evaluate efficacy with respect to joint or skin health or muscle recovery. Although detailed safety evaluations were not included in these 6 studies, adverse effects were monitored, and the study authors concluded in all 6 studies that the intervention products were well tolerated, with no significant difference in the incidence, severity, or type of adverse effects reported between the placebo and intervention groups (Llopis-Miró *et al.*, 2012; Schauss *et al.*, 2012; Schwartz and Park, 2012; Di Cerbo *et al.*, 2015; Lopez *et al.*, 2015). In the remaining 4 studies, detailed evaluations of objective safety parameters were conducted, including vital signs, hematology, clinical chemistry, urinalysis, and adverse effects (Kanzaki *et al.*, 2015; Kumar *et al.*, 2015; Lugo *et al.*, 2016). In these 4 studies, a total of 351 adult men and women (40 to 75 years of age) with knee pain or knee osteoarthritis were assigned in a randomized, double-blind, placebo-controlled manner to consume products providing up to 10 g

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<sup>2</sup> The administration protocol is described by Schauss *et al.* (2007) as: "The individual doses of the test product were determined based on each animal body weight and prepared within 2 h of administration as a 0.6% (low), 6.0% (intermediate), and 20% (high) w/v solution in distilled water. All doses were volumetrically equal (100 mL) and administered orally using a stainless steel ball-tipped gavage needle attached to a syringe."

collagen (of various types)/day and/or up to 1,200 mg chondroitin sulfate/day for 91 to 180 days. In 2 studies, the collagen and chondroitin components of these products were described specifically as type II collagen and chondroitin sulfate (Kanzaki *et al.*, 2015; Lugo *et al.*, 2016). In the remaining 2 studies, the products were described only as collagen peptides (derived from porcine skin or bovine bone) (Kumar *et al.*, 2015). Adverse effects also were monitored in these 4 studies, with none attributable to the study products. Any statistically significant changes reported in these parameters remained within normal ranges, and were not considered physiologically relevant.

The IOM established a Recommended Dietary Allowance<sup>3</sup> for protein of 0.8 g/kg body weight for adult males and females, and concluded that there were insufficient data to set a Tolerable Upper Intake Level for total protein or for any of the amino acids (IOM, 2005). In addition, the IOM noted that “the risk of adverse effects resulting from excess intakes of protein from foods appears to be very low at the highest estimated intake[s]” (*i.e.*, up to 142 g/day in adult males) (IOM, 2005). The Joint Expert Committee on Food Additives (JECFA, 1971), the Select Committee on GRAS Substances (SCOGS) (FASEB, 1975), and the Cosmetic Ingredient Review (CIR, 1985) reviewed the safety of oral and dermal exposure to gelatin (*i.e.*, hydrolyzed collagen) and concluded that they had no safety concerns with respect to the use of the compound as a food or cosmetic ingredient. Gelatin has been consumed as a food ingredient in the U.S. for over 165 years, with no documented evidence of adverse effects attributable to gelatin (FASEB, 1975). Gelatin also is used as an excipient in many oral and parenteral pharmaceutical formulations, with allergic responses reported rarely, and associated primarily with parenteral exposure (FASEB, 1975; Rowe *et al.*, 2009). The SCOGS (FASEB, 1975) concluded that “there is no evidence in the available information on gelatin that demonstrates or suggests reasonable grounds to suspect a hazard to the public when it is used at levels that are now current or that might reasonably be expected in the future”.

The results of several recently published studies also provide supportive evidence of safety for the intake of type II collagen under the proposed uses of Peptan II as a food ingredient. Increased absolute kidney weight (attributed to very high dietary protein intakes) was reported compared to control animals in a study in which juvenile rats were given diets providing 16,600 mg porcine-derived collagen (1,000 to 30,000 Da; molecular type not reported)/kg body weight/day for 4 weeks (Wu *et al.*, 2004), and in ovariectomized spontaneously hypertensive stroke-prone rats given drinking water providing approximately 10,000 mg low-molecular weight collagen (predominantly type I)/kg body weight/day for 20 weeks (Watanabe-Kamiyama *et al.*, 2010)<sup>4</sup>. No additional compound-related effects were reported in either study. Wu *et al.* (2004) described the observed increase in kidney weight in animals consuming 16,600 mg collagen/kg body weight/day for 4 weeks as a hypertrophic response to extremely high dietary protein intake, and noted that this

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<sup>3</sup> The average daily dietary nutrient intake level sufficient to meet the nutrient requirement of nearly all (97 to 98%) healthy individuals in a particular life stage and gender group (IOM, 2005).

<sup>4</sup> Relative kidney weights were not reported in either study, nor were histopathological examinations of the kidneys conducted.

level of intake (*i.e.*, approximately 996 g collagen/day for a 60-kg human) would be highly unlikely in human consumers of products containing collagen. Watanabe-Kamiyama *et al.* (2010) also attributed the reported increase in kidney weight to very high dietary protein intake, with the administered dose of 10,000 mg collagen/kg body weight/day equivalent to approximately 600 g collagen/day for a 60-kg human. In neither of these 2 studies was the observed increase in kidney weight attributed to a toxicological effect of collagen peptides specifically; rather the increase in kidney weight was attributed to extremely high dietary protein intakes (Wu *et al.*, 2004; Watanabe-Kamiyama *et al.*, 2010). No adverse compound-related effects were reported in several other studies in which mice or piglets were given 200 mg collagen peptides/kg body weight/day, or rats were given 1,660 mg/kg body weight/day, for 6 to 9 weeks (Wu *et al.*, 2004; Matsuda *et al.*, 2006; Tanaka *et al.*, 2009).

No compound-related adverse effects were reported in a series of studies in healthy adults, post-menopausal women, adults with joint pain, or adults with osteoarthritis, type 2 diabetes, mild hypertension, or pressure ulcers, who consumed products providing up to 40 g hydrolyzed collagen (of various molecular types)/day (alone or with low doses of other cartilage-derived compounds including hyaluronic acid or chondroitin sulfate) for up to 20 months (Barnett *et al.*, 1998; Lee *et al.*, 2006; Clark *et al.*, 2008; Benito-Ruiz *et al.*, 2009; Cúneo *et al.*, 2010; Zhu *et al.*, 2010; Trč and Bohmová, 2011; Bruyère *et al.*, 2012; Llopis-Miró *et al.*, 2012; Schauss *et al.*, 2012; Schwartz and Park, 2012; Kouguchi *et al.*, 2013; Jiang *et al.*, 2014; Wang *et al.*, 2015; Inoue *et al.*, 2016; Lugo *et al.*, 2016). These studies, and the endogenous nature of collagen peptides, support the safety of type II collagen intake from the proposed uses of Peptan II (providing up to 3.0 g type II collagen/day).

In a recently published animal study of chondroitin sulfate-sodium, a NOAEL of 1,000 mg/kg body weight/day was reported based on a lack of compound-related adverse effects on mortality, clinical signs, body weight, food consumption, hematology, clinical chemistry, urinalysis, organ weights, histopathology, or gross necropsy in Sprague-Dawley rats administered chondroitin sulfate-sodium by gavage at doses of 0, 30, 300, or 1,000 mg/kg body weight/day for 90 days (Miraglia *et al.*, 2016). The results of a bacterial reverse mutation assay<sup>5</sup>, a chromosomal aberration assay<sup>6</sup>, and an *in vitro* mammalian cell gene mutation assay<sup>7</sup> also were reported in this publication to be negative (Miraglia *et al.*, 2016).

Hathcock and Shao (2007) conducted a risk assessment of dietary supplementation with chondroitin sulfate in humans, and determined an observed safe level (OSL) of 1,200 mg/day (the highest dose identified). Hathcock and Shao (2007) noted that this dose is not a true NOAEL, and that the true lowest-observed-adverse-effect level is likely to be much higher. In a recently

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<sup>5</sup> Test conducted using *S. typhimurium* (TA1535, TA1537, TA98, and TA100) and *E. coli* (WP2uvrA), with up to 5,000 µg chondroitin sulfate sodium/plate, with or without metabolic activation.

<sup>6</sup> Test conducted *in vitro* using Chinese hamster ovary cells, with chondroitin sulfate sodium at concentrations of ≤5,000 µg/mL, with or without metabolic activation.

<sup>7</sup> Test conducted using mouse lymphoma L5178Y cells, with chondroitin sulfate sodium at concentrations of ≤5,000 µg/mL, with or without metabolic activation.

published Cochrane Review (Singh *et al.*, 2015) on the efficacy and safety of chondroitin (including chondroitin sulfate) in subjects with osteoarthritis, the authors conducted a meta-analysis including 43 randomized, controlled intervention studies in which a total of 9,110 adult men and women ( $\geq 18$  years of age) with varying degrees of osteoarthritis consumed chondroitin or chondroitin sulfate (at doses of 200 to 2,000 mg/day), alone or in combination with glucosamine (or placebo). Singh *et al.* (2015) concluded that “chondroitin did not result in statistically significant numbers of adverse events or withdrawals due to adverse events” compared to placebo or active control products.

In 10 additional human studies, a total of 4,343 adults with osteoarthritis or chronic knee pain consumed up to 1,200 mg chondroitin sulfate/day (with or without glucosamine sulfate or hydrochloride) for 3 to 24 months (Mazières *et al.*, 2007; Zegels *et al.*, 2013; Hochberg *et al.*, 2014, 2016; Fransen *et al.*, 2015; Provenza, *et al.*, 2015; Herrero-Beaumont *et al.*, 2016; Lugo *et al.*, 2016; Roman-Blas *et al.*, 2016). In a single-dose study, 24 healthy adult subjects were given 2,400 mg chondroitin in a fasting state (Miralgia *et al.*, 2016). Adverse events were reported with equal frequency in treatment and control groups. The study authors concluded that chondroitin sulfate was well tolerated, and did not raise any safety concerns.

Although no studies designed specifically to investigate the allergenicity of Peptan II or its components were identified, compound-related allergic reactions were reported rarely in the identified authoritative reviews, assessments, meta-analyses, or clinical studies. Hochberg *et al.* (2016) reported 1 allergic reaction among 264 subjects given 1,200 mg chondroitin sulfate and 1,500 mg glucosamine hydrochloride/day for 6 months. Schauss *et al.* (2012) reported 1 mild allergic reaction (considered probably related to treatment) among 40 subjects consuming 2 g BioCell Collagen II/day for 12 weeks. As proteins with molecular weights less than 5,000 Da tend to be poorly allergenic (Kuby, 1997), the partially hydrolyzed proteins present in Peptan II are not expected to be allergenic. However, the potential of hypersensitivity to porcine proteins may exist. Thus, Rousselot recommends that manufacturers of food products containing Peptan II ensure that this product is in compliance with local regulation in force.

The totality of information and the weight of the available evidence reviewed by the Expert Panel, as described herein, was sufficient to support a determination that the intended use of Peptan II at a level up to 4.0% in variety of food products intended for consumption by adults [including active nutrition and nutritionally complete bars, granola bars, enhanced fortified water beverages, sports nutrition gels, fortified flavored milk beverages (excluding milkshakes), enhanced or fortified fruit-flavored beverages, and fortified gummies] (providing up to 1.8 g Peptan II/person/day, including up to 1.3 g type II collagen and 0.5 g chondroitin sulfate/day) is safe and suitable and GRAS. Pivotal supporting data include the following:

- 1) Peptan II is manufactured from porcine trachea cartilage in accordance with a Food Safety system compliant with HACCP principles, using safe and suitable food grade raw materials,

reagents, and processing aids. This process results in a final product that consistently meets specifications and is stable for  $\geq 12$  months under ambient storage conditions.

- 2) Peptan II is a product containing primarily 2 different, unbound components with different structures/chemistry (*i.e.*, chondroitin sulfate and type II collagen). Peptones, gelatin, and various molecular types of collagen are natural components of foods, and are permitted for use as food ingredients in the U.S. Commercial products similar to Peptan II (*i.e.*, derived from animal cartilage), or mixed products containing various molecular types of collagen and/or chondroitin sulfate, are currently marketed in the U.S. as dietary supplements and/or food ingredients.
- 3) Collagen hydrolysates are enzymatically degraded in the gastrointestinal tract to small peptides and amino acid components (Walrand *et al.*, 2008). Peptides and individual amino acids derived from collagen are well absorbed from the gastrointestinal tract and widely distributed in rats, mice, and humans (Iwai *et al.*, 2005; Ohara *et al.*, 2007; Walrand *et al.*, 2008; Zague, 2008; Watanabe-Kamiyama *et al.*, 2010; Wang *et al.*, 2015). Chondroitin sulfate also is absorbed (5 to 15% of an oral dose in humans) as the parent compound and small oligosaccharides and widely distributed (Palmieri *et al.*, 1990; Conte *et al.*, 1991, 1995; Ronca and Conte, 1993; Adebawale *et al.*, 2002; Sakai *et al.*, 2002; Lamari *et al.*, 2006; Miraglia *et al.*, 2016).
- 4) No compound-related adverse effects were reported in rats in an acute or 90-day study of a product similar to Peptan II (containing 10% hyaluronic acid, 60% type II collagen, and 20% chondroitin sulfate) (Schauss *et al.*, 2007). A NOAEL of 1,000 mg/kg body weight/day, the highest dose tested, was reported in the 90-day study. Likewise, no compound-related adverse effects were reported in 10 human studies of cartilage-derived products similar to Peptan II at doses providing up to 10 g collagen peptides and 600 mg chondroitin sulfate/day for up to 180 days (Llopis-Miró *et al.*, 2012; Schauss *et al.*, 2012; Schwartz and Park, 2012; Di Cerbo *et al.*, 2015; Kanzaki *et al.*, 2015; Kumar *et al.*, 2015; Lopez *et al.*, 2015; Lugo *et al.*, 2016).
- 5) Collagen (various molecular types) is endogenous in humans and has a long history of use as an ingredient in foods and pharmaceuticals, with no documented compound-related adverse events, and is a natural component of the diet (JECFA, 1971; FASEB, 1975; CIR, 1985; Rowe *et al.*, 2009). No compound-related adverse effects were reported in mice, rats, or piglets at doses up to 1,660 mg collagen peptides/kg body weight/day (administered for up to 9 weeks) (Wu *et al.*, 2004; Matsuda *et al.*, 2006; Tanaka *et al.*, 2009), or in humans at doses up to 40 g collagen peptides/day, consumed for up to 20 months (Barnett *et al.*, 1998; Lee *et al.*, 2006; Clark *et al.*, 2008; Benito-Ruiz *et al.*, 2009; Cúneo *et al.*, 2010; Zhu *et al.*, 2010; Trč and Bohmová, 2011; Bruyère *et al.*, 2012; Llopis-Miró *et al.*, 2012; Schauss *et al.*, 2012; Schwartz and Park, 2012; Kouguchi *et al.*, 2013; Jiang *et al.*, 2014; Wang *et al.*, 2015; Inoue *et al.*, 2016; Lugo *et al.*, 2016).

- 6) Chondroitin sulfate is endogenous in humans and has a long history of use in dietary supplement products in the U.S., Canada, and the EU. A NOAEL of 1,000 mg chondroitin sulfate-sodium/kg body weight/day (the highest dose tested) was determined based on a lack of adverse effects reported in a 90-day study in rats (Miraglia *et al.*, 2016). The results of 3 *in vitro* genotoxicity studies demonstrate that chondroitin sulfate lacks genotoxic potential (Miraglia *et al.*, 2016). An OSL of 1,200 mg chondroitin sulfate/day was determined by Hathcock and Shao (2007) based on a lack of adverse effects in a number of human studies. No compound-related adverse effects were reported in a Cochrane review of chondroitin sulfate or in 10 additional human studies identified in the literature, at doses up to 1,200 mg/day, consumed for up to 24 months (Mazières *et al.*, 2007; Zegels *et al.*, 2013; Hochberg *et al.*, 2014, 2016; Franssen *et al.*, 2015; Provenza, *et al.*, 2015; Singh *et al.*, 2015; Herrero-Beaumont *et al.*, 2016; Lugo *et al.*, 2016; Roman-Blas *et al.*, 2016).
- 7) Compound-related allergic reactions were reported rarely among the identified authoritative reviews, assessments, meta-analyses, or clinical studies of products similar to Peptan II, collagen (various molecular types), or chondroitin sulfate. As proteins with molecular weights less than 5,000 Da tend to be poorly allergenic (Kuby, 1997), the partially hydrolyzed proteins present in Peptan II are not expected to be allergenic.

## CONCLUSION

We, the undersigned independent qualified members of the Expert Panel, have individually and collectively critically evaluated the data and information summarized above, as well as other data and information that we deemed pertinent to the safety of the proposed uses of hydrolyzed porcine cartilage (Peptan II) for use as a food ingredient in the United States. We unanimously conclude that the proposed uses of Peptan II, produced in accordance with Hazard Analysis and Critical Control Points (HACCP) principles and meeting appropriate food grade specifications presented in the dossier, in a variety of food products intended for consumption by adults [including active nutrition and nutritionally complete bars, granola bars, enhanced fortified water beverages, sports nutrition gels, fortified flavored milk beverages (excluding milkshakes), enhanced or fortified fruit-flavored beverages, and fortified gummies], at levels up to 4.0%, providing up to 1.8 g/person/day, are safe and suitable and Generally Recognized as Safe (GRAS) based on scientific procedures.

It is our opinion that other qualified experts would concur with these conclusions.

(b) (6)

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*28 March 2017*

Date

(b) (6)

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Date

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