

**CARRAGEENAN (INS 407) AND PROCESSED EUCHEUMA SEAWEED (INS 407a)**  
**MONOGRAPH IN RESPONSE TO REQUEST FOR DATA FOR THE 68<sup>TH</sup> MEETING**  
**OF JECFA**

**Prepared by:**

**MARINALG INTERNATIONAL**  
**BRUSSELS, BELGIUM**

**November, 2006**

## Table of Contents

|  | Page |
|--|------|
| Executive Summary  | 4    |
| Part   |      |
| 1. Physicochemical discussion of carrageenan and Processed Eucheuma Seaweed as related to JECFA specifications and toxicological considerations. | 6    |
| 1.1 Carrageenan and PES as Food Additives  | 6    |
| 1.1.1 Carrageenan Chemistry and Structure  | 6    |
| 1.1.2 PES Chemistry and Structure  | 7    |
| 1.2 Carrageenan and PES Stability  | 8    |
| 1.2.1 General Considerations   | 8    |
| 1.2.2 Stability during Manufacturing and Food Production   | 10   |
| 1.2.3 Physical Stability during Digestion  | 14   |
| 1.3 Molecular Weight of Carrageenan and PES  | 15   |
| 1.3.1 The EU Specification and the Measurement Quandary  | 15   |
| 1.3.2 Basics of Polymer Molecular Weight and Molecular Weight Distribution   | 17   |
| 1.3.3 SEC Measurement Systems (All Failed to be validated)   | 18   |
| 1.3.3.1 Overview of Systems and Operating Conditions   | 18   |
| 1.3.3.2 Results  | 20   |
| 1.3.3.3 Future Work on LMT Measurement   | 23   |
| 1.4 Conclusions  | 24   |
| 1.5 References   | 26   |
| 2. A Review of the toxicology literature pertaining to carrageenan and Processed Eucheuma Seaweed published 1997 – 2006.                         | 31   |
| 2.1 Introduction   | 31   |
| 2.2 Short-Term Toxicological Studies   | 34   |

|            |   |    |
|------------|---|----|
| 2.3        | Long-term Toxicity and Carcinogenicity Studies  | 36 |
| 2.4        | Tumor Promotion   | 37 |
| 2.5        | Epidemiology studies  | 42 |
| 2.6        | Tumor Suppression/Anti-tumor Activity   | 43 |
| 2.7        | Immune Response   | 44 |
| 2.8        | Summary and Conclusions   | 46 |
| 2.9        | References  | 47 |
| 3.         | Safety assessment for infants 0-6 months from exposure to carrageenan through infant formulas                                       | 57 |
| 3.1        | Discussion  | 57 |
| 3.2        | References  | 59 |
|            | Overall Conclusions   | 60 |
|            | Appendices  | 60 |
| Appendix 1 | Proposed Specifications for Carrageenan defined as the consolidation of INS 407 carrageenan and INS 407a Processed Eucheuma Seaweed | 62 |
| Appendix 2 | Proposed INS Number for carrageenan defined as the consolidation of carrageenan INS 407 and Processed Eucheuma Seaweed INS 407a.    | 66 |
| Appendix 3 | Poligeenan – A limited use pharmaceutical excipient   | 67 |

## Executive Summary

Carrageenan (INS 407) and Processed Eucheuma Seaweed (PES) (INS 407a) are food additives listed in Table 3 of the Codex General Standard for Food Additives (GSFA). They are permitted for use in the listed categories in Table 3 under the conditions of Good Manufacturing Practices (GMP). The most recent review of carrageenan and PES was performed in 2001 at the 57<sup>th</sup> meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). At that meeting, the JECFA considered the effects of carrageenan on gastrointestinal tract as well as studies on tumor promotion in the rat colon and cell proliferation. The committee concluded that "... the intakes of carrageenan and processed *Eucheuma* seaweed from their use as food additives was of no concern. It therefore allocated a group ADI 'not specified' to the sum of carrageenan and processed *Eucheuma* seaweed."

Most recently, at the request of the Codex Committee on Food Additives and Contaminants (CCFAC), the JECFA included carrageenan and processed Eucheuma seaweed on its priority list for review in June, 2007. The CCFAC requested and the JECFA agreed to undertake a toxicological re-evaluation, including specific data relevant to the safety assessment for infants 0-6 months from exposure through infant formulas, and revision of specifications. (CCFAC, 2006; JECFA, June 2006)

This dossier is submitted in response to the JECFA's request for data. It demonstrates that:

The current JECFA specifications are sufficient to control production of quality carrageenan and PES products while protecting the health and safety of the consumer.

Carrageenan and processed Eucheuma seaweed remain appropriately classified as ADI 'not specified' and for use under conditions of GMP.

Carrageenan remains a suitable component of infant formula for consumption by infants 0 – 6 months.

Since the approval of PES as a food additive, product quality has increased and knowledge of its functionality has expanded to the point where there are many applications where carrageenan and PES can be used interchangeably, or they can be used in combination as a means of cost reduction. Having seriously evaluated this situation, Marinalg International, the organization submitting this dossier, proposes that carrageenan (INS 407) and Processed Eucheuma Seaweed (INS 407(a)) be consolidated under one name, carrageenan, and one INS number, 407. In this regard, a proposed set of specifications reflecting the consolidated product with the name carrageenan is presented for consideration.

This dossier was prepared by Marinalg International, a trade association that represents worldwide producers of seaweed extracts and maintains non-governmental organization status at the Codex. Marinalg International gratefully acknowledges Dr. Samuel Cohen (Professor and Chair, Havlik-Wall Professor of Oncology, Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, Nebraska, USA) and Dr. Nobuki Ito (Professor and President Emeritus, Nagoya City University Medical School, Nagoya, Japan) for evaluating the current toxicology literature for carrageenan and processed Eucheuma seaweed and preparing the toxicology discussion and comments with reference to the use of carrageenan in formula for infants 0-6 months that are presented in the dossier.

## **Part 1. Physico-chemical discussion of carrageenan and Processed Eucheuma Seaweed as related to JECFA specifications and toxicological considerations.**

### **1.1 Carrageenan and PES as Food Additives**

#### **1.1.1 Carrageenan Chemistry and Structure**

Carrageenan is the long chain, water soluble polymer of galactose with the galactose units populated periodically with sulfate groups, i.e. sulfated polygalactans. A more detailed chemical structure is described in the definition section of the JECFA specifications (JECFA 2001). In this description, the prevalent sulfated galactose polymers in carrageenan are designated as kappa, iota and lambda carrageenan. Kappa carrageenan is the polymer consisting mostly of the alternating monomeric units, D-galactose-4-sulfate (1,3 linked) and 3, 6-anhydro-D-galactose (1,4 linked). Iota carrageenan is similar except that the 3,6-anhydrogalactose is sulfated at carbon 2. Between kappa carrageenan and iota carrageenan there is a continuum of intermediate compositions differing in degree of sulfation at carbon 2. In lambda carrageenan the alternating monomeric units are mostly D-galactose-2-sulfate (1,3-linked) and D-galactose -2,6-disulfate (1,4-linked) (JECFA 2001)

Carrageenan has been an item of commerce for more than sixty years, and therefore has attracted numerous published scientific studies. As a result, the science of carrageenan has been well documented. The only addition to the 2001 JECFA definition would be recognizing that food additive carrageenan always contains varying amounts of the precursors to kappa and iota carrageenan (Van de Velde *et al* 2001, 2002, 2005) (Wichmann 2006) (Guibet 2006). These precursors contain a 6-sulfate rather than the 3, 6 anhydro-D-galactose. They are termed mu and nu in the scientific literature (Lahaye 2001). If the amount of precursor is large enough it can impart lambda-like, cold water soluble properties to kappa and iota carrageenans, and in particular to the intermediate compositions referred to in the JECFA definition. (Falshaw *et al* 2001)(Bixler *et al* 2001)

### 1.1.2 PES Chemistry and Structure

PES is composed principally of water soluble molecular carrageenan and up to 15% of insoluble algal cellulose (Bixler 1996) (Phillips 1996) (JECFA 2001b). PES as a food additive may be produced from either dried commercial *Eucheuma cottonii* or *Eucheuma spinosum* seaweeds. If produced from the former seaweed the carrageenan is primarily kappa, and if it is produced from the latter seaweed the carrageenan is primarily iota.

The algal cellulose is an integral part of PES because of the way it is produced (Gunning *et al* 1998). In producing PES the seaweed is soaked in 2N KOH at 70C. This process extracts and removes most of the cellular DNA, proteins, fats, sugars and heavy metals, but leaves behind (after water washing) the carrageenan, algal cellulose, and those harmless impurities, e.g. crude protein and fats that were not removed during extraction . At this point in the process the carrageenan and cellulose contain about 55% moisture. When these insoluble components are dried (12% H<sub>2</sub>O), there is 9 to 11% cellulose when the seaweed is *E. cottonii* and 5 to 7% when the seaweed is *E. spinosum*.

Carrageenan (E-407) contains a negligible amount of algal cellulose (<0.2%) because of the way it is processed from the seaweeds. The carrageenan is extracted from the dried seaweed with dilute alkali at about 100C. This produces a dilute solution of carrageenan (1 to 1.5%) from which the algal cellulose and other minor insoluble components are removed by filtration.

The practical way of estimating the cellulose and other impurities in PES is by measuring the AIM or acid insoluble matter. This standard measurement is applied to a number of polysaccharide food additives for the purpose of controlling adulteration. In the case of PES, the dry product is dispersed in hot, concentrated sulfuric acid (JECFA

2001b). The quantity of washed and dried residue is the AIM. Since this dried residue can be retained, it can be analyzed. The reported analyses yield AIM compositions for PES that on average contain 90% cellulose with the remaining 10 % consisting of carrageenan that was probably physically blocked from dissolving in the hot acid plus xylan, crude protein and crude lipid (Bixler 1996) (Phillips 1996). Since carrageenan (E-407) contains so little AIM it also contains negligible amounts of xylan, crude protein and crude lipid.

Based on over 15 years of use of PES as a food additive, there is overwhelming proof that it behaves simply as pure carrageenan diluted with cellulose. The algal cellulose has all the chemical and physical characteristics of that of cotton linters (Hoffmann 1996), and is a mixture of crystalline and amorphous cellulose (Phillips 1996)). PES as a food additive must and does meet all of the specifications for carrageenan (E-407) as a food additive except for the AIM being in excess of 2%. Marinalg has proposed in the Overview that E-407 and E-407a be brought together under one JECFA specification. The highlights of the integrated specification are shown in Appendix 1. The proposal for a single INS number is presented in Appendix 2.

## **1.2 Carrageenan and PES Stability**

### **1.2.1 General Considerations**

For purposes of discussion in this Section, the term carrageenan is used to represent both carrageenan (INS-407) and Processed Eucheuma Seaweed (INS 407a). The principal components of PES are kappa and iota carrageenans that are subject to the same stability considerations whether in INS-407 or INS-407a. While detailed *in vitro* measurements of the pH stability of PES versus carrageenan have not been done, there is no sound reason to suspect there would be any difference. The same is true for *in vivo* stability in the human GI tract. Carrageenan is classified as a soluble fiber for human nutrition purposes. The cellulose in PES is also very stable in the pH range of

processed foods and in the GI tract. Cellulose is classified as an insoluble fiber for human nutrition purposes.

The retained structural combination of carrageenan and cellulose in PES renders this carrageenan component slightly more stable than carrageenan that has already been extracted and separated from the cellulose, but this is far from being significant enough to give carrageenan and PES separate consideration with respect to stability.

The content, references, and conclusions in the Carrageenan Monograph prepared and submitted by the International Food Additives Council and Marinalg International dated November, 1997- (“1997 monograph”) and reviewed by the JECFA at its 51<sup>st</sup> meeting are reiterated to commence the discussion of current literature.

As we have seen from earlier discussion, carrageenan comprises high molecular weight sulfated polygalactans derived from a number of species of red seaweeds of the class *Rhodophyceae*. It is important to maintain this high molecular weight, because it is key to all carrageenan’s various functions and value to the food industry. Commercial carrageenan must have a minimum viscosity of 5 cps measured at 1.5% at 75C (Appendix 1), which corresponds to a weight average molecular weight (Mw) in the range of 100,000 to 150,000 daltons depending on the associated cations in the sample. Commercial carrageenan average molecular weights are generally in the range 200,000 to 800,000 Daltons. Different carrageenan types have normal Gaussian molecular weight distributions, even though their average molecular weights may be different. These distributions include the small amounts of lower molecular weight materials (less than 50,000 daltons), naturally present as part of the growing live seaweed.

Although carrageenan is very stable and resistant to degradation, some care has to be taken to preserve molecular chain length for effective functional and economic application in food products. Degradation of carrageenan is avoided during all steps of manufacture and food preparation, the primary concerns being its stability at pH values below 6 and temperatures above 40C. However, strict control of exposure to adverse

pH, temperature, and time minimizes degradation and preserves its high molecular weight.

Carrageenan must not be confused with the deliberately acid-hydrolyzed sulfated polygalactose product, “poligeenan”, which is not an approved food additive, has a molecular weight of 10,000 – 20,000 Daltons and viscosity well below 1 cps at 1.5% and 70C and is used solely in clinical applications such as barium enemas for X-ray of the human GI tract. A thorough discussion on poligeenan is detailed in Appendix 3. This section will demonstrate that the high molecular weight of carrageenan is readily preserved both before food preparation and after consumption as food.

### 1.2.2 Stability during Manufacture and Food Production

Seaweeds used for the production of carrageenan are dried without delay to avoid “composting” via enzymatic and microbial degradation. Dried seaweeds are very stable, primarily due to the high and saturated sea salt content. Good post-harvest practices are well documented (Blakemore, 1989.).

Seaweed cultivation is also subject to degradative diseases such as “ice-ice”, the whitening and softening of part of the cultivar. Work by Mendoza *et al* (2002) concluded that healthy kappa carrageenan plants yielded Mw of 717,000 Daltons, reduced to 30,000 Daltons by “ice-ice”, thought to be caused by enzymes liberated by pathogenic bacteria. However, the disease was estimated to affect less than 1% of the weight of an infected plant. Consequently, considering that over 99% of the seaweed plants are free of ice-ice, the total significance of ice-ice is very low at less than 0.01% on a carrageenan basis. The carrageenan industry uses strict raw material testing criteria to control carrageenan quality.

Extraction of carrageenan is carried out under well-controlled alkaline conditions for maximum stability and minimal degradation. Although some thermal degradation is

expected during any heat treatment, in the case of carrageenan extraction the impact on Mw and molecular weight distribution is minimal and insignificant. As thermal degradation also reduces most functionalities, and hence commercial value, the industry takes all steps to minimize the impact through strict control of extraction parameters such as pH, temperature, and time.

Isolated carrageenan powders are slightly alkaline at about pH 8 to 10 to maintain this highly stable condition during storage. When carrageenan is stored under cool dry conditions it is stable in excess of two years.

Concerns have been expressed about the accidental production of low molecular carrageenan that could end up in food products for human consumption (Tobacman, 2001). Such an accident would quickly be detected through normal quality control procedures as functionalities would be significantly impacted, and, in line with GMP, the adulterated product would be removed from the production line for waste disposal. It should also be noted that carrageenan comprising low molecular weight material would not pass routine quality control specifications, both by the manufacturer and the customer.

Although several process alternatives are available for carrageenan extraction, for example, alcohol precipitation, gel-pressing, PES processing, the final molecular weights and distributions remain similar, which, if for no other reason, must be done in order to meet food processor requirements for functionality.

Carrageenan applications in food products cover a wide range of functional uses to include stabilization, gelation, and thickening. Applications can be segmented in several ways such as milk versus water, low solids versus high solids, gelling versus non-gelling, hot versus cold, etc. However, when using carrageenan the major considerations are the processing conditions: pH, temperature, and time exposures.

Proper control of well-established and accepted food processing conditions and protocols ensures that minimal carrageenan degradation will occur and that the carrageenan will function as expected and desired. If excessive degradation were to take place, the carrageenan will either not function at all, or, at best, result in unacceptable food product characteristics. If carrageenan depolymerization does occur, it is principally by cleavage of the glycosidic bonds, which would eventually result in permanent loss of basic functionalities, most notably rheological behavior, thus rendering the carrageenan useless for its intended purpose. Glycosidic bond cleavage occurs more or less randomly so during depolymerization the Gaussian Mw distribution is maintained, and no bimodal distribution is noted (Lecacheux, D., *et al*, 1985, covered in 1997 Monograph).

The rate of depolymerization of carrageenan depends on the process conditions (pH, temperature, time). However, molecular conformation also plays an important role, the gel state being much more stable than the sol state for both kappa and iota carrageenans. The repeating 2-sulphate of iota carrageenan gives added protection against glycosidic cleavage compared to kappa carrageenan which has no 2-sulphate units. Lambda carrageenan has few 3,6-anhydrogalactose units to adversely influence glycosidic cleavage, and consequently is the most stable of the carrageenans. Food processors are very much aware of the care needed when applying carrageenan under acid conditions, but also very much aware of the long shelf life of carrageenan-based gelled products such as up to 12 months for ready-to-eat desserts at pH 4. In this case, shelf life is limited by factors other than carrageenan, for example, deterioration of color.

The presence of other food components can have significant positive impacts on carrageenan stability, particularly those that interact (for example, structural cross-linking) with the carrageenan (for example, proteins, locust bean gum, konjac), or tighten the carrageenan gel structure (for example, salts, high solids). As most degradation studies on carrageenan have been carried out on carrageenan alone, these results and conclusions about carrageenan stability can be considered as the worst case scenario.

At pH values above 6, carrageenan is highly stable, even at very high temperatures (for example autoclaving). Heating profiles for both pasteurization and ultra high temperature (UHT) processing have essentially no effect on molecular weight.

At pH values below 6, care has to be taken to minimize carrageenan exposure to processing conditions. For example, production of ready-to-eat water gel desserts at pH 4 usually involves combinations of (a) pre-solubilization of all the ingredients at neutral pH, (b) adding the acid immediately prior to filling and (c) rapid cooling to a gel to realize the already-mentioned gel-mode stability. The resulting dessert gel is stable for up to twelve months. The shelf-life of these desserts is normally 2-3 months, which is far short of the carrageenan gel stability, but as indicated earlier, the shelf-life is more affected by other factors such as color deterioration.

The absolute lowest molecular weight capable of forming a recognizable gel (very weak and only detected by rheological profile) is about 40,000 Daltons for kappa carrageenan, and, more importantly, the critical molecular weight needed to show useful textural differences in foods begins at about 180,000 Daltons (Rochas, *et al*, 1990, covered in 1997 Monograph). Below about 180,000 Daltons, carrageenan gels lack structural integrity, the short molecular chain lengths interfering with the creation of an effective three-dimensional network. The same structural logic applies to effective protein interactions. Consequently, the carrageenan specification of minimum 5 cps (equivalent to 100,000 to 150,000 Daltons) is a highly appropriate one.

Work by Marrs (1998) on the stability of carrageenans to processing concluded that there is no reason to suppose that extensive degradation of carrageenan takes place during the processing of foods. This, together with the published information on gel stability at pH 4, suggests that there is no compelling reason for introducing a specified limit on the low molecular weight fraction for carrageenan in food products. Marrs endorsed the previous comments about structural interactions with proteins and other ingredients leading to additional acid stability, the need for strict control of

processing conditions, and the better stability of iota carrageenan over kappa carrageenan.

Marrs also concluded that commercial carrageenan can have broad molecular weight distributions with as much as 25% below 100,000 Daltons. However, we must note that the Mw distribution of poligeenan is significantly different than the component of carrageenan below 100,000 Daltons. As stated previously, carrageenan has Gaussian Mw distribution, and the distribution of its low molecular section will be a continuance of this normal distribution, with the bulk of this fraction being at the upper end of the range and above 50,000 Daltons. However, the Mw of poligeenan is in the range 10,000 to 20,000 Daltons, all of which will be below 100,000 Daltons or even 50,000 Daltons, essentially all below 25,000 Daltons, and most below 15,000 Daltons. Consequently, the lower molecular weight component of carrageenan must never be considered as or compared with poligeenan. The levels of lower molecular weight material in carrageenan with similar profile to poligeenan are extremely low.

Carrageenan stability and degradation technology has not advanced significantly since 1997, with only a few endorsements of prior positions and opinions. This lack of additional work is most likely due to the fact that carrageenan stability and degradation kinetics were studied in great depth prior to 1997, the conclusions of these publications being summarized in the 1997 monograph.

Carrageenan remains a proven stable food additive with the necessary specifications, controls, and knowledge in place for effective and safe application in food products. The minimum viscosity of 5 cps is a simple, but very effective specification for assuring a safe additive for food use.

### 1.2.3 Physical Stability During Digestion

Carrageenan is relatively stable during digestion. This is due to three primary factors; gel-mode conformation of carrageenan resisting acid hydrolysis in the stomach,

added protection of structural interactions with proteins in the stomach, and very low enzymatic and microbial breakdowns in the intestine.

Carrageenan has low caloric value and limited fermentation in the large intestine. However, the limited fermentation that occurs has demonstrated positive effects of carrageenan as a soluble dietary fiber, specifically blood cholesterol, plasma cholesterol, lipid levels, and hypoglycemia, (Smit, 2004, Panlasidui *et al*, 2003, Tunland, *et al*, 2002, and Hoebler, *et al*, 2000).

The feeding study and faecal analysis by (Uno *et al* (2001) on rats concluded minimal degradation of lambda carrageenan from 832,000 Daltons to 782,000 Daltons after one day in the digestive system, and to 718,000 Daltons after two days. This endorses the low caloric value of carrageenan and the minimal fermentation of carrageenan in the large intestine. The data from this work clearly refutes many if not all of the presumptive theories of Tobacman (2001).

### **1.3 Molecular Weight of Carrageenan and PES**

#### **1.3.1 The EU Specification and the Measurement Quandary**

Though the JECFA has not specifically raised the matter of establishing a molecular weight specification for carrageenan and PES, Marinalg International would be remiss not to discuss the matter, since the European Commission has implemented such a specification, and they are represented at CCFA.

In April 2003, The European Union adopted a new specification for carrageenan. It was in addition to the simple water viscosity specification that serves as a surrogate for an average molecular weight. The new specification requires knowledge of molecular weight distribution of carrageenan. In particular, the specification called for carrageenan intended for human consumption to contain not more than five percent of

its molar mass that is less than 50,000 Daltons in molecular weight (referred to henceforth in this monograph as the low molecular weight tail or the LMT).

The adoption of a new EU specification was initiated by an opinion from the European Commission's Scientific Committee for Food (EC-SCF) (European Commission 2003) This arose from the EC's review of a published review of past toxicology studies of carrageenan and poligeenan by Joanne Tobacman (Tobacman 2001). This and other Tobacman papers are more thoroughly discussed in the toxicology section of this dossier. As will be seen in that section, the Tobacman papers inappropriately attribute poligeenan study results to carrageenan and they evaluate effects from routes of administration that are irrelevant to the ingestion of carrageenan.

In March of 2003 the EC-SCF recommended the LMT specification be adopted if its measurement were feasible (European Commission 2003). Since that time the major carrageenan producers of the world under Marinalg International, their regulatory affairs association, have been working, not only to determine feasibility, but under the EU dictum, to solve the measurement problem. Six laboratories have participated in this work, some within carrageenan company laboratories and one within the laboratories of a large carrageenan user that had the necessary facilities and personnel to operate them, the remainder being done by outside laboratories under contract to those carrageenan companies who did not have in-house capabilities. Without going any further it must be emphatically stated that as of the date of this monograph no method meeting laboratory GLP for method validation has been found. It should also be noted that although the European Commission implemented a low molecular weight tail specification in the purity criteria for carrageenan and PES in April 2004, a validated analytical method does not exist.

To commence the project, scientists from the carrageenan industry gathered to consider measurement methods that might work. Some preliminary work had been done to see if a more sophisticated water viscosity measurement than in the current specification could be used to measure the LMT, This approach had been strongly

encouraged by EC-SCF and JECFA representatives at CCFAC 2005 as a way of keeping testing costs within reason. However, this quickly had to be discarded for lack of positive results. It was then decided that a method based on size exclusion chromatography (SEC) and light scattering (LS) had the best chance of success.

Before reviewing the results obtained to date, it will be necessary to review some of the basics of the technology involved in SEC-LS (Wu 2004). It is not the purpose of this monograph to delve deeply into a technology, but this particular use of SEC-LS has been fraught with unresolved problems on which measuring a very important regulatory specification hangs. Therefore, some discussion of the technology is necessary.

The Marinalg team has discussed this issue with numerous SEC-LS experts who believed that the problem would be easy to solve, but this has been shown not to be the case. The methods built around SEC-LS work well if the purpose is to determine average molecular weight or a roughly accurate molecular weight distribution. However, when challenging the methods to come up with accurate and repeatable numbers on a very small part of the carrageenan molecular weight distribution (the LMT) they fail.

### 1.3.2 Basics of Polymer Molecular Weight and Molecular Weight Distribution

Carrageenan is a polysaccharide with a distribution of molecular weights either within the growing seaweed from which it is obtained or after being extracted and purified for human food use. The usual way to characterize a physical property such as water viscosity of carrageenan with its distribution of molecular weights is by an average molecular weight. There is an excellent correlation between viscosity and Mw, when both are measured with the carrageenan in the “free draining coil” state (Tanford 1961).

Depending on whether one is averaging the molecular weight by the number of chains of a certain size (chain length) or the weight of the chains of that size, one determines the number average molecular weight ( $M_n$ ) by the former and the weight

average molecular weight ( $M_w$ ) by the latter (Tanford 1961).. For a polymer with a molecular weight distribution that is approximately Gaussian like carrageenan, the polydispersity index (PDI) or  $M_w/M_n$  is approximately 2.5 or less. PDIs of about 3.0 and above must be reached before significant abnormal distributions can be visually detected from spectral analysis (Wu 2004)

Depending on the method used to extract carrageenan from seaweed,  $M_w$  will vary from 200,000 to 800,000 (Hoffmann *et al* 1996) (Uno *et al* 2001b) (Blakemore 1989). Nevertheless, the PDI will be generally in the range 1.5 to 2.5. This means the LMT should be approximately proportional to  $M_w$ . Unfortunately this approximation cannot be relied upon to predict the LMT with sufficient precision, so it must be measured. The challenge, therefore, is putting these reasonably well behaved characteristics of carrageenan to use in developing a method for measuring the LMT.

### 1.3.3 SEC Measurement Systems (All Failed to be Validated)

#### 1.3.3.1 Overview of Systems and Operating Conditions

The SEC-LS methods studied by the Marinalg team consisted of pumping a heated (40 to 65C) dilute solution of carrageenan through one or more heated size exclusion chromatography columns in order to separate the carrageenan into a continuous stream of virtual fractions based on their molecular size. The fractions exiting the column passed through a refractive index (RI) detector to measure the concentration of carrageenan in the fractions and through a LS detector to measure a signal that can be software manipulated into  $M_w$  and  $M_n$ .

Two variations on LS were studied: (a) multi-angle laser light scattering (MALLS) and (b) a combination of low angle laser light scattering (LALLS) and right angle laser light scattering (RALLS). Although both light scattering techniques claim to measure absolute molecular weight, LALLS/RALLS comes closer to achieving  $M_w$  directly (Wu 2004b, Viscotek 2004)

So as not to leave the impression that only these gross equipment differences need be considered, here are other differences encountered between laboratories in the Marinalg study that must be taken into consideration (Wu 2004):

- GPC column selection including the number of columns
- Operational conditions, e.g. temperature, concentration, purification, flow rate, cations
- Value used for  $dn/dc$
- Software, including extrapolations and corrections

It would also be misleading to assume that had there been better control over these variables one or more laboratories could have been validated to measure the LMT. The problem is much bigger than this.

The most significant problem arises from the fact that all of the LS systems give light scattering signals that fail below 100,000 daltons (MALLS) or 50,000 daltons (LALLS/RALLS). Therefore, measurement of the LMT depends on mathematical extrapolation of LS data into the region of interest (less than 50,000 daltons). None of these extrapolation techniques is particularly good; although because of its lower Mw cutoff, LALLS/RALLS does a slightly better job. A second source of error of equal or greater consequence is the necessity of picking a somewhat arbitrary baseline for the RI (fraction concentration) data.

One laboratory employed SEC and RI coupled with inductively coupled plasma (ICP) to assure only carrageenan, and not diluents such as maltodextrin, was being measured. For this method that used no LS, Mw had to be determined indirectly. The exit times from the SEC column were determined for a series of pullulan standards of known Mw and low PDI (<1.1). They were then used to correlate elution volume with Mw (Uno 2001b). It has not yet been proven that pullulan calibration is a good model for carrageenan calibration, but no carrageenan standards exist today.

### 1.3.3.2 Results

The six participating laboratories tested thirteen commercially produced carrageenan samples in a Round Robin mode. The samples included high and low molecular weight kappa and iota carrageenans as well as kappa and iota PES. Table 1 below displays the key results for two of these samples that highlight some of the problems encountered with the methods employed.

One sample is a low molecular weight kappa carrageenan taken from the lot of carrageenan used for a new rat feeding study, the toxicology results of which are presented later in this monograph. This carrageenan has a water viscosity of 8 cps. Since this is above the 5 cps JECFA limit, it is perfectly acceptable for human food use. The other carrageenan sample is a high molecular weight kappa carrageenan used commercially in a wide variety of food applications.

The results in Table 1 show the software output data of most interest: Mw and LMT. These values are then averaged and 95% confidence intervals are calculated and presented. Mn values are not presented for all tests; because all LS methods give a poor estimate of this parameter. However, a few PDI values (Mw/Mn) are given for illustration.

The SEC-MALLS results show mostly inter-laboratory variability; although some repeats by the same lab are shown. For SEC-LALLS/RALLS all of the work was done in one laboratory as was the work on SEC-RI-ICP. Because of the mix of inter-and intra-lab results its hard pinpoint one methods being better than another. Mw by SEC-MALLS and SEC-LALLS/RALLS are in reasonable agreement, but Mw values by SEC-RI-ICP are twice or more than those of the other methods, again raising questions about the use of pullulan standards as surrogates of carrageenan standards. There is a technique known as Universal Calibration that can be used to convert a pullulan calibration into a carrageenan calibration (Kostanski *et al* 2004). The Marinalg group

applied this technique to the Uno data, and while it partially closed the gap with the LS data, a large gap remained.

Table 1 Molecular Weight Data

| Carrageenan                    | Laboratory                     | Lower Mw Kappa |                | Higher Mw Kappa  |                |             |
|--------------------------------|--------------------------------|----------------|----------------|------------------|----------------|-------------|
| Test Method                    | #                              | Mw             | LMT (%)        | Mw               | LMT (%)        |             |
| <b>SEC/RI/MALLS</b>            | 6                              | 256,000        | 5.20           | 613,000          | 0.00           |             |
|                                | 4                              | 253,000        | 9.04           | 662,000          | 1.64           |             |
|                                | 6                              | 238,000        | 8.65           | 595,000          | 0.00           |             |
|                                | 5                              | 195,000        | 8.75           |                  |                |             |
|                                | 5                              | 190,000        | 12.05          | 549,000          | 9.00           |             |
|                                | 1                              | 189,000        | 7.40           | 406,000          | 0.45           |             |
|                                | 1                              | 188,000        | 7.80           | 395,000          | 1.30           |             |
|                                | 4                              |                |                | 561,000          | 5.00           |             |
|                                | <b>Average</b>                 |                | <b>215,571</b> | <b>8.41</b>      | <b>540,143</b> | <b>2.48</b> |
|                                | <b>Standard Deviation</b>      |                | <b>29,476</b>  | <b>1.91</b>      | <b>94,673</b>  | <b>3.10</b> |
| <b>95% Confidence Interval</b> |                                | <b>21,835</b>  | <b>1.41</b>    | <b>70,133</b>    | <b>2.30</b>    |             |
| <b>SEC/RI/LALLS/RALLS</b>      | 3                              | 261,000        | 4.30           | 585,000          | 0.00           |             |
|                                | 3                              | 259,000        | 6.54           | 563,000          | 5.40           |             |
|                                | 3                              | 258,000        | 7.20           |                  |                |             |
|                                | 3                              | 252,000        | 4.90           |                  |                |             |
|                                | <b>Average</b>                 |                | <b>257,500</b> | <b>5.74</b>      | <b>574,000</b> | <b>2.70</b> |
| <b>95% Confidence Interval</b> |                                | <b>3,287</b>   | <b>1.15</b>    | <b>15,245</b>    | <b>3.74</b>    |             |
| <b>SEC/RI/ICP</b>              | 2                              | 632,000        | 2.21           | 1,259,000        | 2.20           |             |
|                                | 2                              | 559,000        | 2.42           | 1,042,000        | 2.50           |             |
|                                | <b>Average</b>                 | <b>595,500</b> | <b>2.32</b>    | <b>1,150,500</b> | <b>2.35</b>    |             |
|                                | <b>95% Confidence Interval</b> | <b>50,585</b>  | <b>0.15</b>    | <b>150,371</b>   | <b>0.21</b>    |             |

The LMTs by laboratory #2 (SEC-RI-ICP) are well below the EU LMT specification and for the two measurements presented, the LMT Confidence Limits are quite low. This is to be expected. If Mw is incorrect and too high, the LMT would be expected to be very low. These measurements meet the EU specification for both samples, but there is probably an artifact in the data (the pullulan standards problem),

For a time the SEC-LALLS-RALLS method was showing promise in measuring the LMT with reasonable accuracy. One set of tests that upset this false confidence was to spike a high Mw carrageenan with various percentages of poligeenan. SEC-LALLS/RALLS did not even come close to closing the material balance on the poligeenan and carrageenan.

The results presented here emphasize that no light scattering technique can pass validation. This conclusion stems from analysis of much more data than is appropriate to show in this Monograph. To see more of the data than could be shown here see (Marinalg Website 2004)

LS techniques are excellent R&D tools, for example, for following reactions, monitoring compositions, etc., but cannot accurately and consistently measure the LMT in line with a pass/fail specification of no more than 5.0% below 50,000 Daltons.

#### 1.3.3.3 Future Work on LMT Measurement

It does not appear that any further work with SEC-LS is justified unless major improvements in equipment were forthcoming and unless there was a regulatory need based on sound science to do so. Discussions with equipment developers do not indicate the breakthroughs needed to measure LMT with accuracy are on the horizon. Furthermore, the high cost of SEC-LS or SEC-RI-ICP equipment and the need for highly qualified technicians to operate the equipment, tend to defeat the JECFA's goal of keeping cost of analysis and risk to safety in balance.

As the toxicology parts of this monograph will clearly show, all of this may be moot given the understanding of the current toxicology data base and the results of a new rat - 90 day feeding study. In this regard, we believe that the LMT specification established by the EC may prove to be superfluous.

## **1.4 Conclusions– Carrageenan and PES**

The chemistry and structure of carrageenan has been so thoroughly studied and published over the last sixty years that little that is new has shown up in the decade since the 1997 Carrageenan Monograph was prepared. The only advance worth noting is the knowledge that kappa and iota carrageenan as food additives have always contained varying amounts of their precursors present in the seaweeds from which they are extracted. If the amount of precursor is high enough in the intermediate compositions of kappa and iota then they can behave in a similar manner to lambda with respect to cold water solubility. However the level of precursor in the carrageenan in live seaweed does not exceed about 30%.

PES kappa and iota have gained commercial prominence since the 1997 Monograph. It is now generally agreed that the only significant difference between PES and carrageenan in the kappa and iota forms is the presence of 8 to 15% algal cellulose in the former. This cellulose shows up in the AIM purity criterion that for carrageenan is less than 2%. All other purity criteria in the JECFA monographs for these two food additives are identical, and there is no indication that producers of PES have any problem meeting these criteria with current GMPs. The two additives also share a group ADI “not specified”.

Even with their extremely close relationship with respect to food additive properties and toxicology, Carrageenan (INS-407) and PES (INS-407a) still must appear on ingredients labels (except in the US) as two different additives because of the “a” subscript on PES. It is time this unnecessary administrative burden is eliminated, and the two additives are consolidated under the name carrageenan with one INS number: 407. A proposed set of specifications for the consolidated product is presented in Appendix 1 for the JECFA’s consideration.

The stability of carrageenan against hydrolysis during manufacture and food processing under GMP conditions continues to be supported by the scientific literature. These conditions include minimum processing times and temperatures and optimum pH to assure carrageenan's proper function in foods. There is certainly no evidence that poligeenan is produced in extracting carrageenan from seaweeds. Though both are sulfated polygalactoses, poligeenan is clearly distinguishable from the food additive carrageenan. Under GMP there is no economic or performance reason to believe that carrageenan can or should be contaminated with poligeenan. As for carrageenan breakdown after being eaten, at least one new study supports the contention that carrageenan undergoes negligible degradation as it traverses the GI tract.

The carrageenan industry has had a working group under Marinalg International attempting for three years to measure the EU's April 2004 LMT specification for carrageenan (less than 5% of the molar mass less than 50,000 daltons molecular weight). The three methods most extensively studied failed to yield LMT results of sufficient accuracy and reproducibility to be validated as a regulatory specification. One thing to keep in mind is that in none of the work done by this group was there any strong evidence of EU LMT specification being exceeded for carrageenans for food use.

It is believed that the new 90-day rat feeding study using an 8 cps carrageenan (current limit 5 cps) should answer the questions raised on LMT and thus negate the need to undertake further work to find a method that is practical with respect to cost and that can be validated, an exercise which to date has proved to be extremely difficult.

The specifications as presently written are sufficient to support the continued use of carrageenan and PES in food without concern for safety.

## **1.5   References**

Bixler, H.J. (1996) Recent developments in manufacturing and marketing carrageenan. *Hydrobiologia* **326/327**: 35-57

Bixler, H.J., R. Falshaw and K. Johndro (2001) Structure and performance of commercial kappa-2 carrageenan extracts. II. Performance in two simulated dairy applications. *Food Hydrocolloids* **15**: 619-630

Blakemore, W.R. (1989) Post Harvest Treatment and Quality Control, *Proceedings of the Regional Workshop on Seaweed Culture and Marketing, FAO Development Project*, Editors Adams & Foscarini, Suva, Fiji, 48-52.

European Commission (2003) Opinion of the Scientific Committee on Food on Carrageenan *SCF/CS/ADD/EMU/199 Final* 1-8

Falshaw, R., H.J. Bixler, K Johndro (2001) Structure and performance of commercial kappa-2 carrageenan extracts. I. Structure analysis. *Food Hydrocolloids* **15**: 441-452

Guibet, M., N. Kervarec, P. Boulenger, J Mazoyer, A. Critchley and W. Helbert (2006) Enzymatic Analysis of  $\lambda/\kappa$  Hybrid Carrageenan in eds. G.O. Phillips and P.A. Williams *Gums and Stabilisers for the Food Industry* **13**: 52-60.

Gunning, A.P., P. Cairns, A.R. Kirby, A.N. Round, H.J. Bixler and V.J. Morris (1998) Characterising semi-refined carrageenan networks by atomic force microscopy *Carbohydrate Polymers* **36**: 67-72

Hoebler, C., *et al* (2000) Supplementation of pig diet with algal fibre changes the chemical and physicochemical characteristics of digesta. *J. Sci. Food Agric.* **80**: 1357-1364

Hoffmann, R.A., A.L. Russell and M.J. Gidley (1996) Molecular weight distribution of carrageenan: Characterisation of commercial stabilizers and effect of cation depletion on depolymerisation . in eds. G.O. Phillips, P.A. Williams, and D.J. Wedlock, *Gums and Stabilisers for the Food Industry* **8**: 137-148.

JECFA (2001) Compendium of food additive specifications: Carrageenan *Food and Nutrition Paper* FAO Rome **52 Add 9**: 13 – 20

JECFA (2001b) Compendium of food additive specifications: PES *Food and Nutrition Paper* FAO Rome **52 Add 9**: 81 - 87

Kostanski, L.K., D.M. Keller and Archie E. Hamielec (2004) Size-exclusion chromatography-a review of calibration methodologies *J. Biochem.Biophys.Methods* **58**: 159-186.

Lahaye, M. (2001) Chemistry and physico-chemistry of phycocolloids. *Cah. Biol. Mar.* **42**:137-157

Lecacheux, D., *et al* (1985) Molecular Weight Distribution of Carrageenans by Size/Exclusion Chromatography and Low Angle Laser Light Scattering *Carbohydrate Polymers* **5**: 423-440 .

Marinalg Website (2004 )[http://www.marinalg.org/papers/c\\_papers.htm](http://www.marinalg.org/papers/c_papers.htm) Marinalg Position Paper Regarding European Molecular Weight Specification for Carrageenan (E-407) and Processed Eucheuma Seaweed (E-407a)

Marrs, W.M. (1998) The Stability of Carrageenans to Processing in eds. P.A. Williams and G.O. Phillips *Gums and Stabilisers for the Food Industry* **9**: 345-357.

Mendoza, W.G., *et al* (2002) Chemical and gelling profile of ice-ice infected carrageenan from *Kappaphycus striatum* (Schmitz) Doty "sacol" strain (Solieriaceae, Gigartinales, Rhodophyta) , *Journal of Applied Phycology*, **14**: 409-418

Panlasigui, L.N., *et al*, (2003) Blood cholesterol and lipid-lowering effects of carrageenan on human volunteers, *Asia pacific J Clin Nutr* 2003: **12 (2)**: 209-214.

Phillips, G.O. (1996) The chemical identification of PNG-carrageenan. in eds. G.O. Phillips, P.A. Williams, and D.J. Wedlock, *Gums and Stabilisers for the Food Industry* **8**: 403-423.

Rochas, C., *et al*, (1990) Role of the Molecular Weight on the Mechanical Properties of Kappa Carrageenan Gels, *Carbohydrate Polymers* **12**: 255-266

Smit, A.J.(2004) Medicinal and pharmaceutical uses of seaweed natural products: A review *Journal of Applied Phycology* **16**: 245-262

Tanford, C.(1961) Physical Chemistry of Macromolecules *J. Wiley and Sons, Inc., New York* Chapter 3.

Tobacman, J.K.(2001) Review of Harmful Gastrointestinal Effects of Carrageenan in Animal Experiments *Environmental Health Perspectives* **109 (10)**: **983-994**

Tungland, B.C., *et al* (2002), Nondigestible Oligo- and Polysaccharides (Dietary Fiber): Their Physiology and Role in Human Health and Food., *Comprehensive Reviews in Food Science and Food Safety*, **3**: 73-92.

Uno, Y, *et al* (2001) Molecular Weight and Fecal Excreted Quantity of Carrageenan Administered to Rats in Blended Feed *Japanese Journal of Food Chemistry* **8 (2)**: 83-93

Uno, Y., T. Omoto, Y. Goto, I. Asai, M. Nakamura and T. Maitani (2001b) Molecular weight distribution of carrageenans studied by combining gel permeation/inductively coupled plasma (GPC/ICP) method. *Food Additives and Contaminants* **19 (9)**: 763-772

Van de Velde, F., H.A. Peppelman, H.S. Rollema, and R.H. Tromp (2001) The structure of  $\alpha$ - $\beta$  hybrid carrageenans. *Carbohydrate Research* **331**: 271-283

Van de Velde, F., S.H. Knutsen, A.I. Usov, H.S. Rollema and A.S. Cerezo (2002)  $^1\text{H}$  and  $^{13}\text{C}$  high resolution NMR spectroscopy of carrageenans: applications in research and industry. *Trends in Food Science and Technology* **13**: 73-92

Van de Velde, F., A.S. Antipova, H.S. Rollema, T.V. Burova, N.V. Grinberg, L. Pereira, P.M. Gilseman, R.H. Tromp, B. Rudolph and V.Y. Grinberg (2005) The structure of  $\alpha$ - $\beta$  hybrid carrageenans II Coil-helix transition as a function of chain composition. *Carbohydrate Research* **340**: 1113-1129

Villanueva, R.D., W.G. Mendoza, M.R.C. Rodriguez, J.B. Romero and M.N.E. Montano (2004) Structure and functional performance of gigartinacean kappa-iota carrageenan and solieriacean kappa iota blends. *Food Hydrocolloids* **18**: 283-292

Viscotek (2004) Theory: Principles of Triple Detection GPC/SEC

Wichmann, J. T.M.I.E. Christensen, and J. de Vries (2006) Distribution of Kappa- and Iota-Carrageenan Structures in Hybrid Carrageenans in eds. G.O. Phillips and P.A. Williams *Gums and Stabilisers for the Food Industry* **13**: 61-70

Wu, C. (2004) Handbook of Size Exclusion Chromatography and Related Techniques *Marcel Dekker, Inc. New York* Chapter 1 Introduction to Size Exclusion Chromatography, E. G. Malawer and L. Senak

Wu, C. (2004b) Handbook of Size Exclusion Chromatography and Related Techniques  
*Marcel Dekker, Inc. New York* Chapter 10 Size Exclusion Chromatography of Polyvinyl  
Alcohol and Polyvinyl Acetate, D.J. Nagy

## **Part 2. A review of the Toxicology literature pertaining to carrageenan and Processed Eucheuma Seaweed published 1997 – 2006.\***

\*(prepared by Dr. Samuel Cohen (Professor and Chair, Havlik-Wall Professor of Oncology, Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, Nebraska, USA) and Dr. Nobuki Ito (Professor and President Emeritus, Nagoya City University Medical School, Nagoya, Japan))

### **2.1 Introduction**

Carrageenan is a high-molecular-weight (weight average > 100,000 Da.) sulfated polygalactan derived from several species of red seaweeds of the class Rhodophyceae. It is used for texturizing and stabilizing processed foods and has no nutritive value. It was reviewed for the Joint FAO/WHO Expert Committee on Food Additives (JECFA) at their 57<sup>th</sup> meeting in 2001, and the literature was extensively reviewed by us in a previous publication (Cohen and Ito, 2002). The present review will focus primarily on publications and data available since 1997, after a brief summary of previous issues.

The most common forms of carrageenan are designated as κ-, ι-, and λ-carrageenan. Carrageenan is the aforementioned high molecular weight galactose polysaccharides extracted from specific seaweeds for use by the food industry. Carrageenan has often been confused with poligeenan. Poligeenan involves a different manufacturing process. It utilizes carrageenan as the starting material which is exposed to intentional extensive acid hydrolysis at high temperatures (>80°C), resulting in significantly smaller sulfated galactose polymers with an average molecular weight of approximately 15,000 Da. (see Appendix 3), (International Food Additives Council and Marinalg International, 1983; 1991; 1997; IARC, 1983; USAN, 1988). Poligeenan is used to a minor extent in the pharmaceutical industry. The distinction between carrageenan and poligeenan is essential because their physical and toxicological properties differ considerably. In addition, in contrast to carrageenan, poligeenan has no food performance properties, even at concentrations as high as 10% in aqueous

food preparations, whereas carrageenan will function in food at concentrations as low as 0.01%. Consequently, poligeenan has no application as a food additive.

Though of no toxicological significance, carrageenan contains a negligible amount of the low-molecular-weight polymers. Depending on the analytical method used, the low-molecular-weight forms are <12% of the total composition of the commercial carrageenan used in foods. Because carrageenan is extracted from seaweeds under alkaline conditions, degradation to smaller polymerized polysaccharides is avoided. As long as the pH is maintained above 6.0, carrageenan is stable to heat processing. Once carrageenan is in the gel configuration, as is the case for its use in food systems, the carrageenan becomes highly resistant to degradation, even under more acidic conditions, such as occur in the stomach (see Section 1.2.3).

Carrageenan ingested in the gel form (either as a homogenous carrageenan gel or one consisting of a carrageenan /protein gel from a meat or a dairy food) is also stable to the conditions of passage through the digestive tract (Abraham et al., 1972; Benitz et al., 1973; Arakawa et al., 1988; Weiner, 1988). It has been reported that < 6% of the excreted carrageenan is below 100,000 Da. and < 1% is below 50,000 Da. Because of its large molecular weight, carrageenan remains within the lumen of the digestive tract and is not absorbed (Weiner, 1988; 1991). Thus, there are no systemic effects of carrageenan following ingestion by rats, mice, or monkeys. In guinea pigs, it has been reported that carrageenan is absorbed to a slight extent (Engster and Abraham, 1976). This observation is based on demonstration of staining of the macrophages of the lamina propria of the cecum and proximal colon by toluidine blue or by positive iron staining following administration in the drinking water of iron-labeled carrageenan. However, even in guinea pigs, there is no evidence that the absorbed material in the intestinal tract is distributed systemically because there is no excretion in the urine (International Food Additives Council and Marinalg International, 1991; 1997). Generally, it can be concluded that orally administered carrageenan is not absorbed through the gut and is not metabolized to lower molecular weight material.

Systemic administration of carrageenan has been shown to have various effects, particularly on the immune system (IARC, 1983; Weiner, 1991; JECFA, 1999). However, because carrageenan is not absorbed from the gastrointestinal tract, studies involving administration systemically are not relevant to a risk assessment of carrageenan exposure from foods or beverages.

Based on these basic properties of carrageenan, there are several observations which must be followed in any evaluation of literature regarding the potential safety of carrageenan from food and beverage exposures:

1. Carrageenan is a high-molecular-weight substance with well defined specifications (a.k.a. purity criteria).
2. In its commercially utilized form, carrageenan is not degraded in the intestinal tract.
3. Carrageenan administered in food and beverages is not absorbed from the gastrointestinal tract.
4. Studies involving poligeenan, a low molecular-weight substance (historically referred to as “degraded carrageenan” and which is produced by the acid hydrolysis of carrageenan at  $>80^{\circ}\text{C}$  for extended periods of time to produce smaller oligosaccharides), are not relevant to the biological or toxicological evaluation of carrageenan.
5. Experiments involving systemic administration (subcutaneous, intraperitoneal, intravenous) are not relevant to biological or toxicological evaluation of orally administered carrageenan.
6. *In vitro* studies evaluating carrageenan are not relevant to the biological or toxicological properties of orally-administered carrageenan.
7. The relationship between the molecular weight and concentration of the test material needs to be considered. The concentration in diet, especially in drinking water, can greatly affect the acceptability of the administered substance by the test animal, as well as affecting availability of water and nutrition.

In publishing a study in the scientific literature, it is essential to fully disclose the characteristics of the carrageenan being administered, including type and viscosity, the dose and form in which it is being administered, the route of administration, and an evaluation of the specific bioassays being utilized to assess the biological or toxicological properties of carrageenan. A comparison to commercially utilized carrageenan and poligeenan should be provided.

## **2.2 Short-term Toxicological Studies**

For orally administered carrageenan, the only short-term toxicological issue that has been identified in the past is the question of whether it is capable of producing damage to the gastrointestinal tract, particularly the intestine (see Cohen and Ito, 2002). As mentioned above, in guinea pigs, there is evidence that carrageenan can be transported across the intestinal epithelium, being taken up in the macrophages of the lamina propria, but not leading to systemic exposure. Others have claimed that there is an inflammatory response produced in the large intestine, possibly including ulceration, following oral administration of carrageenan. These previous studies have had several complicating features, such as excessive doses in the diet or water (> 5%), and not adequately defining the composition of the carrageenan with respect to its molecular weight and distribution.

To better evaluate the possibility of the inflammatory effect on the gastrointestinal tract of rats, food-grade  $\lambda$ -carrageenan was administered to rats for 90 days, with a thorough evaluation of the gastrointestinal tract histopathologically, including evaluation of the intestine utilizing a Swiss-roll methodology (Weiner et al., 2006). The specific type of k-carrageenan was chosen as the test article because it was at the lower end of the viscosity specification (8 cps by the standard water viscosity test versus the JECFA lower limit of 5 cps). The sample used had a relatively high percentage of low molecular weight polymer (an average of 7% < 50,000 Da). The Swiss-roll method was used because it permits more extensive evaluation of the

intestine than routine sections. Even with this amount of low molecular weight material, there was no evidence of erosions, ulcerations, inflammation, regeneration, hyperplasia, or any other abnormalities of the entire gastrointestinal tract (Weiner et al., 2006). This study addressed the concerns of previous studies by utilizing commercial food-grade  $\lambda$ -carrageenan at just above the minimum viscosity JECFA specification of 5cps. The test material, as already noted, was at 8cps by this specification test. In deference to the purity criteria established by the European Commission, the low molecular weight tail was measured using various analytical methods that reflect the best available current technology. It was administered at reasonable levels of the diet (= 5%), and there was full histopathologic evaluation of the gastrointestinal tract. The study was performed under Good Laboratory Practices (GLP).

The difficulty evaluating some studies relating to the toxicological effects of carrageenan on the gastrointestinal tract is illustrated in a recent series of publications by Donnelly et al., (2004a, 2004b, 2004c, 2005) utilizing  $\lambda$ -carrageenan. The form of the carrageenan utilized was purchased commercially from a chemical supplier, but did not address the specifications of food-quality carrageenan. The typical viscosity of carrageenan that is sent to suppliers such as Sigma is 500-1000 cps. The  $\lambda$ -carrageenan was administered in the drinking water at concentrations of 1% and 4%. These test conditions compromise the study because at 1% concentration, the solution would have been extremely thick, and at 4% it must have been more like paste. This would be expected to influence the water consumption and water availability for the animals. Such an effect was observed. In these studies, there was mention of inflammatory changes in the large intestine (see below), which very likely influenced the outcome. The high concentration and abnormal texture likely contributed to the induction of colitis, and raises serious concern regarding the relevance of these studies to human exposures. Colitis has not been reported in association with carrageenan ingestion in humans.

### **2.3 Long-term Toxicity and Carcinogenicity Studies**

Cohen and Ito (2002) reviewed in detail the carcinogenicity testing of carrageenan, and concluded that there was no evidence of carcinogenicity of carrageenan in animal models. Likewise, they concluded that there was no evidence of tumor-promoting activity by carrageenan in animal models (see below). No additional long-term carcinogenicity studies have been performed since the previous review.

However, Tobacman (2001, 2003) has published a series of articles questioning the interpretation of the carcinogenicity of carrageenans and other safety issues. She raises questions with respect to carcinogenicity, tumor promotion, inflammatory and immune effects. However, in her publications, she interchangeably uses the studies related to carrageenan and poligeenan, and all of the conclusions that she is drawing regarding the carcinogenicity of carrageenan are based on studies with poligeenan. This was pointed out in a letter to the editor by Carthew (2002). Tobacman suggested that there is extensive breakdown of carrageenan to small-molecular-weight products which could be carcinogenic. The evidence is strongly contrary to that conclusion. The effects related to tumor promotion are addressed below. Her conclusions related to effects on inflammation and immune effects are all based on studies involving systemic administration of carrageenan or poligeenan, and as described above, are not relevant to the interpretation of the safety of orally administered carrageenan. Orally administered carrageenan is not digested into small-molecular-weight forms, it is not absorbed or distributed systemically, and shows no evidence of toxicological effects up to a dose of 5% of the diet or drinking water (Cohen and Ito, 2002; IFAC, 1997; IARC, 1983; JECFA, 1999).

In separate articles, Tobacman (1997) and Tobacman and Walters (2001) describe changes of filament structure and formation of inclusions (probably lysosomes) in mammary myoepithelial cells exposed *in vitro* to  $\alpha$ -carrageenan. Tobacman also published two abstracts describing additional studies utilizing the mammary myoepithelial cells (Tobacman, 1999; Tobacman and Zhila, 2002), but these have not

been published as full manuscripts. In these studies, the myoepithelial cells are exposed to  $\beta$ -carrageenan *in vitro*. Although the concentrations are reasonable (.01%), these studies have two major difficulties regarding relevance to humans following oral administration. To begin with, the exposure is *in vitro*, which, for extrapolation to an *in vivo* setting, would assume systemic exposure. Systemic exposure does not occur following oral administration. Furthermore, the changes that are seen in these studies are basically those related to cell death. There is the possibility that *in vitro*, carrageenan and other large-molecular-weight polysaccharides can alter the surface structure leading to cell death. The changes in the myofilaments are merely a reflection of that process, as are the changes in the lysosomes described in the abstracts. The relevance of these findings to carcinogenesis also is questionable. Myoepithelial cells are a normal component of the breast ductule, lying beneath the ductular epithelial cells and resting on the basement membrane (Rosen and Oberman, 1993). By definition, ductal carcinoma *in situ* involves ductules that have lost the myoepithelial cells, and have only epithelial cells present (Rosen and Oberman, 1993). This does not imply invasion, as suggested by Tobacman (1997, 1999), since this phenomenon occurs in an *in situ* (noninvasive) form of breast carcinogenesis, as well as in the invasive disease. Furthermore, there is no evidence to suggest that loss of myoepithelial cells is causative in the progression from normal to invasive cancer.

## **2.4 Tumor Promotion**

At the last review by JECFA of carrageenan, there was some concern raised regarding possible tumor-promoting activity of carrageenan. As discussed in detail by Cohen and Ito (2002), the studies in which this concern was raised had serious flaws and did not indicate any evidence of tumor-promoting activity for the gastrointestinal tract or any other tissues. Subsequent to this review, some additional studies have been performed to address this issue.

Hagiwara et al. (2001) observed no evidence of tumor-promoting activity following administration of carrageenan after an initiating dose of 1, 2-dimethylhydrazine

(DMH) in male F344 rats. Although no evidence of promoting activity was found with carrageenan, there was no statistically significant evidence of tumor-promoting activity in a positive control utilizing cholic acid as the tumor promoter. Thus, interpretation of this study must be made with caution.

The laboratory of Dr. Corpet published the results of the studies utilizing administration of a carrageenan gel in rats, utilizing aberrant crypt foci in the colon as the marker for promoting activity (Corpet et al., 1997; Taché et al., 2000). In the previous review (Cohen and Ito, 2002), the latter study had not yet been published, but was discussed in considerable detail based on information provided by Dr. Corpet. In brief, there were serious questions raised regarding the validity of these studies, largely because of the marked variation in aberrant crypt foci in the various control groups between the different studies (Cohen and Ito, 2002). Additional shortcomings to the experiments by Corpet and his colleagues include: small numbers of animals utilized in each group; a short experimental treatment period without tumors as an end point; marginal statistical significance in the presence of a highly variable parameter (number of crypts/foci), and there was actually an inhibitory effect on the number of aberrant crypt foci, although the size of the foci was marginally increased at the highest dose. More importantly, these investigators were unable to repeat their initial results in a subsequent experiment, which has now been published (Taché et al., 2000), utilizing conventional rats compared to human-flora-associated-treated rats. Our conclusion of the initial study suggesting that there was not tumor-promoting activity is that it was based on an inappropriate interpretation of the data. Taking all of the studies together, there clearly was no evidence of tumor promoting activity. If one accepts the conclusions of the investigators, themselves, however, one is still left with no relevance to humans, since they did not see any effect utilizing human flora in the rats.

More recently, Donnelly et al. (2004a; 2004b; 2004c, 2005) have reported a series of experiments suggesting a synergistic effect between co-administration of N-methyl-N-nitrosourea (MNU) and  $\beta$ -carrageenan. These investigators utilized metallothionein immunohistochemical positivity in aberrant crypt foci in mouse colon as

the biomarker for an effect. Although these investigators reported an effect in the animals administered MNU plus carrageenan in various administration protocols, there was no effect seen with carrageenan alone. Although a synergistic effect was suggested by these authors between MNU and carrageenan in this model, there are several reservations to these studies which require further investigation.

As mentioned above, the composition of the  $\lambda$ -carrageenan utilized for these studies had a considerably higher viscosity than used (or accepted within specifications) for food-grade carrageenan, the viscosity probably being close to 700-800 cps per regulatory viscosity test. At this high molecular weight, high viscosity level, it is likely that at the 1% and 4% concentrations in the drinking water utilized in their experiments, there would be a reduction in availability of free water to the animals. The authors describe in their studies a decrease in fluid intake at the 4% carrageenan concentration, although not reported at the 1% level. No information regarding water excretion in the urine or composition of the feces is provided. Since AIN-76A diet was used in these studies, much of the water consumed by these animals would usually be absorbed, since animals administered this diet tend to have extremely small, compact, dry feces.

Utilization of MNU as the carcinogen also raises serious questions. To begin with, there is no description of the frequency of preparation of the MNU. Because of its high reactivity in aqueous solutions, it is essential that it be prepared fresh before each use and administered immediately. If not, the results could be seriously skewed, as has previously occurred utilizing MNU in studies of urinary bladder carcinogenesis in rats (Hicks et al., 1975; West et al., 1994).

Assuming that the MNU was freshly prepared, the route of administration also raises serious concerns. In the studies by Donnelly et al (2004a, 2004b, 2004c, 2005), the MNU is administered intraperitoneally. The colon cancer model utilizing MNU utilizes an intra-rectal route of administration rather than intraperitoneal (Cohen et al., 1980; Narisawa et al., 2002). There is no clear evidence in the literature that i.p.-injected MNU would actually produce colon cancer.

More importantly, however, are the consequences of i.p. administration of MNU. MNU is a highly-reactive chemical that produces a severe corrosive and consequent inflammatory response in the tissue wherever it is injected. By injecting it into the peritoneum, it is likely that peritonitis would result. This is not described in the reports by Donnelly et al., but it does not appear to have been evaluated (Donnelly et al., 2004a, 2004b, 2004c, 2005). A proper evaluation requires sacrifice of the animals within a few days of the administration, with histopathologic evaluation. If peritonitis was induced, this would greatly affect the normal function of the gastrointestinal tract, especially the intestine.

Further complicating the interpretation of the results is the finding of inflammation of the intestinal mucosa, including ulceration (Donnelly et al., 2004a, 2004b, 2004c, 2005). As described above, food-grade carrageenan does not produce colitis, and certainly not ulceration (Cohen and Ito, 2002; Weiner et al., 2006). This raises concern regarding the material that was administered, and certainly complicates extrapolation from the animal model to humans. The authors also mention literature regarding systemic influences of carrageenan on inflammation, but again, this is confusing the consequences of systemic administration versus oral administration.

In addition, the doses of both MNU and carrageenan used in these studies were extremely high. The carrageenan by itself had no effect on the metallothionein indices, even though there was apparent mucosal injury as described by the authors. Given the theoretical basis for this assay, any inflammatory process in the colon should give rise to an increase in metallothionein-stained foci since this is a reflection of increased spontaneous mutations arising in increased proliferating mucosa (Jasani et al., 1998; Cook et al., 2000). Certainly, if there is ulceration present, there will be a consequent regenerative proliferative process.

In addition to these difficulties, there are additional difficulties in interpreting the results of these studies. In the paper in the *British Journal of Cancer* (Donnelly et al.,

2005), they indicate that there were no statistically significant differences in large crypts, only in smaller crypts. Other studies have shown that large aberrant crypt foci are likely the ones that are most meaningful with respect to the carcinogenic process (Papanikolaou et al., 2000). Furthermore, there was no difference in the results with carrageenan whether the carrageenan was administered for one week, one week repeated three times, or continuous administration during the entire 20 weeks of the experiment. In addition, there was no evidence of a dose response, the results actually somewhat higher at 1% than at 4% concentrations of the drinking water.

In conclusion, given all of these difficulties in interpretation, extrapolation to a conclusion of a tumor-promoting effect in humans is highly unlikely and not scientifically supported.

Suzuki et al. (2000) described an inhibition of gap-junctional intercellular communication in rat liver epithelial cells following exposure to  $\lambda$ -carrageenan. The specifics of the carrageenan were not defined, other than stating that it was  $\lambda$ -carrageenan. Based on the results of this *in vitro* assay, the authors conclude that the substance could have tumor-promoting activity *in vivo*. In addition to the various reservations expressed regarding the concordance between results in this *in vitro* assay and *in vivo* effects, and particularly in trying to relate the results of these *in vitro* studies to human carcinogenicity (or promoting activity), there are several other difficulties specifically in evaluating carrageenan in this assay. Most importantly, it is an *in vitro* assay. Orally administered carrageenan does not result in systemic exposure, therefore it would not have the possibility of influencing cells other than the gastrointestinal tract. Furthermore, the concentration of the carrageenan in this study was extremely high compared to what could be attained even within the lumen of the gastrointestinal tract given human exposures. Gap-junctional intercellular communication inhibition, like tumor promotion as defined *in vivo*, empirically is a threshold phenomenon.

In the specific study with carrageenan, the interpretation is made even more difficult by the fact that the change was quite transient and mild (Suzuki et al., 2000).

There was only a mild effect after fifteen minutes, but the intercellular communication level had returned to normal by one hour. Furthermore, there was no evidence of a change in connexin 43 localization or phosphorylation, which nearly always occur if the compound also shows promoting activity *in vivo* in rodent studies. Most importantly, many substances that are positive in this *in vitro* assay are not known to be related to an increase in human carcinogenesis or are thought to be preventive, such as retinoids (Rivedal et al., 1994; Mikalsen and Sanner, 1993; Ruch et al., 1987; Rutten et al, 1988). Thus, the predictive value of this assay for human carcinogenesis is highly questionable.

In conclusion, based on a variety of types of assays, there continues to be no convincing evidence of tumor-promoting activity of the gastrointestinal tract in animal models.

## **2.5 Epidemiology Studies**

Since the 1998 review of carrageenan by the JECFA, there have been no epidemiology studies of carrageenan and its possible relationship to cancer or other diseases in humans. The only literature in the past decade even remotely related to this issue was a series of three publications by Tobacman et al. (2000; 2001; 2002). These studies, however, are seriously flawed. They are based entirely on a comparison of gross consumption of carrageenan in the United States with national breast cancer incidence rates. Their analysis assumes an average consumption by all individuals in the United States, an assumption that is clearly not justifiable, as has been discussed at length regarding a variety of alternative methods for assessing consumption (Munro and Danielewska-Nikiel, 2006). Furthermore, as indicated by the authors, utilizing national breast cancer incidence rates cannot provide any adjustment for confounding factors such as age at first birth, age at menstrual onset, family history, obesity, or exogenous hormone exposure. Furthermore, they do not make any adjustment for the increased diagnostic sensitivity over the past three decades of mammography. To conclude that there is a correlation between carrageenan consumption and breast cancer incidence

cannot be justified. Not only are the data seriously flawed and limited, but there are several arguments that can be made specifically against the conclusions. There is considerable evidence over the past decade that fibers of several kinds do not correlate with breast cancer incidence in women despite previous claims that increased fiber ingestion protected against breast cancer (Willett et al., 1992). Furthermore, Tobacman and her colleagues describe a positive correlation for several other fibers, including agar, alginate, gum arabic, and locust bean. Three of these (agar, gum arabic, and locust bean) have been thoroughly evaluated in carcinogenicity assays by the National Toxicology Program and found to be negative (Melnick et al., 1983).

Toxicological consequences of carrageenan exposure in humans have been difficult to evaluate. However, there is no description in the literature, either anecdotal or otherwise, of colitis secondary to carrageenan exposure, except for one case (see below) of alleged allergy-related intestinal abnormalities (Tarlo et al., 1995).

In summary, there is no epidemiologic evidence for a carcinogenic, tumor-promoting, or inflammatory effect of carrageenan in humans.

## **2.6 Tumor Suppression/Anti-tumor Activity**

During the past decade, there have been a number of reports suggesting anti-tumor activity by both  $\lambda$  and  $\kappa$  carrageenan. Although some of these studies have been performed using *in vivo* cancer models, most have involved *in vitro* studies. Zhou et al. (2004; 2005; 2006) have evaluated high-molecular-weight carrageenan, as well as, low-molecular-weight oligosaccharide fragments utilizing the S180 and H22 tumor models. These tumor models involve an ascites tumor model, and involve intraperitoneal injection of the carrageenan or smaller molecular weight oligosaccharide fragments. The model itself is of limited usefulness with respect to predicting anti-tumor activity in humans, and certainly the intraperitoneal route of administration of the test articles, including carrageenan, are not relevant to oral exposure of carrageenan.

Other studies have utilized only degraded carrageenan, low-molecular-weight oligosaccharide fragments, for evaluation. For example, Haijin et al. (2003) used  $\lambda$ -carrageenan which had been enzymatically degraded. Interestingly, utilizing these small fragments orally administered, they showed an inhibition of sarcoma 180 tumor in mice, suggesting that some of the substance was absorbed and systemically distributed. Again, use of small-molecular-weight oligosaccharides has no relevance to oral exposure of large-molecular-weight food-grade carrageenan.

Several investigators have utilized *in vitro* tumor models for evaluating degraded carrageenan oligosaccharide fractions (Haijin et al., 2003; Lambrecht et al., 1998; Liu et al., 2000; Yuan and Song, 2005). None of these *in vitro* assays are relevant to oral exposure to carrageenan.

## **2.7 Immune Response**

Carrageenan has substantial effects on the immune system when administered systemically or in the mouse footpad model. Many of these models have been utilized for examination of basic immune processes. However, these all involve systemic administration, and are not relevant to oral exposure of the large-molecular-weight carrageenan (Cohen and Ito, 2002).

Some recent studies have extended previous effects of systemic administration of carrageenan or of degraded carrageenan to oligosaccharides. For example, the publication by Li et al (2004) involved administration of degraded carrageenan to oligosaccharides, and also involved administration to irradiated mice. Yamada et al., (1997) showed anti-HIV effects, but again, this involved degraded carrageenan (depolymerized), and also utilized an *in vitro* model. Carlucci et al. (1999) showed an anti-herpetic effect, but this also was an *in vitro* evaluation.

An effect on peritoneal macrophages was also demonstrated for both carrageenan and lipopolysaccharide (LPS), but this involved i.p. injection. Abe et al.

(2002) showed the presence of liver injury with specific activation of natural killer cells and natural killer T-cells, but again, this involved intraperitoneal administration of carrageenan, at quite high doses. None of these studies involving systemic administration have relevance to oral exposure to food-grade carrageenan.

In the past few years, there have been studies suggesting an effect on the immune system following oral administration of carrageenan (Frossard et al., 2001; Tsuji et al., 2003). Unfortunately, in most of these studies, the specifics of the administered carrageenan are not provided. Frossard et al. (2001) showed that oral administration of high doses of carrageenan to mice presensitized against BLG anaphylaxis, but only if the mice were presensitized with the combination of carrageenan and BLG. It did not work if presensitized with carrageenan or BLG alone. Since they were administered simultaneously, there may have been an effect of the carrageenan on the structure of BLG or even its availability. Similarly, Tsuji et al. (2003) also showed a suppression of the allergic reaction when low doses of carrageenan were administered mice. It was  $\lambda$ -carrageenan, and it suppressed both antibody production and mitogen-induced T-cell proliferation. LPS also had a similar effect, but apparently by a different mechanism. Nevertheless, the author suggested that low-dose  $\lambda$ -carrageenan as a dietary supplement might be useful for prevention and/or improvement of allergy-related diseases.

There is little evidence in humans with respect to an effect of oral exposure to food-grade carrageenan on the immune system. However, there is one anecdotal report in the literature (Tarlo et al. 1995) reporting a case of anaphylaxis secondary to exposure to a barium enema. Based on allergy skin test and RAST evaluation, the authors concluded that the allergic reaction occurred due to carrageenan and not other substances present in the barium enema. Furthermore, when the patient was placed on what was purported to be a carrageenan-free diet, the patient's previous gastrointestinal symptoms abated. The report indicates the use of sodium carrageenan in the barium enema. Given that it is *Marinalga* membership that produces carrageenan, processed *Eucaema* seaweed, and *Poligeenan*, the industry knows that *Poligeenan*, not

carrageenan, was the material used in the barium enema. The term “poligeenan” was not in general use internationally at the time of the reported incident of anaphylaxis. Furthermore, the properties of carrageenan do not impart the functional characteristics necessary for an effective barium enema. See Appendix 3 for a discussion on the required properties for a barium enema as it relates to the use of carrageenan vs. poligeenan. In the Tarlo et al. (1995) paper, the carrageenan used for the skin test is not detailed. Furthermore, the skin test which was claimed to be positive was actually evaluated as “borderline,” and in some studies, this limited response (2mm) would be considered a negative response. Furthermore, there are no prior or subsequent case reports of an allergic reaction to carrageenan in humans or in animals.

## **2.8 Summary and Conclusions**

In our previous review of carrageenan (Cohen and Ito, 2002), we evaluated older studies in considerable detail and concluded there was no evidence of carcinogenic, tumor-promoting, or mitogenic effects secondary to oral exposure to high-molecular-weight food-grade carrageenan. More recent studies have further evaluated this, but many have significant difficulties as detailed above. Given the lack of an inflammatory response utilizing food-grade carrageenan, the lack of genotoxic effects of carrageenan, and the relatively low levels present in the diet, one can feel confident that dietary carrageenan does not pose a carcinogenic risk to humans. Furthermore, additional studies evaluating possible gastrointestinal inflammation support the conclusion that this is not likely to occur in humans. Studies evaluating possible effects on the immune system are extremely limited, largely because of the use of either systemically-administered carrageenan or use of degraded, low-molecular-weight forms of oligosaccharides derived from carrageenan. None of these studies have relevance to oral exposure to carrageenan in humans. The recent subchronic dietary study in rats of  $\lambda$ -carrageenan whose molecular weight was near the lower JECFA limit as determined by water viscosity (8cps vs. the 5 cps limit) found no effects on the gastrointestinal tract using extensive evaluation methods (Weiner et al, 2006).

For the future, we strongly recommend that certain minimum information and details be provided regarding studies with carrageenan so that misleading information and, more importantly, misleading interpretations, are not allowed to pollute the scientific literature. At the very least, the chemical composition of the carrageenan is required, distinction of food-grade, high-molecular-weight carrageenan from low-molecular-weight, poligeenan and derived oligosaccharides need to be distinguished, as well as oral administration versus systemic administration or *in vitro* studies. When administering the carrageenan orally, it is essential that it be provided in a form that does not interfere with availability of water, such as can occur when too high of a concentration is utilized in the drinking water. Carrageenan is a natural food substance which has been used in the diet around the world for centuries. Scientific evaluation strongly supports the conclusion that it is safe for human consumption.

## **2.9 References:**

Abe, T., Kawamura, H., Kawabe, S., Watanabe, H., Gejyo, F., and Abo, T. Liver injury due to sequential activation of natural killer cells and natural killer T cells by carrageenan. *J Hepatology*, **36**: 614-623, 2002.

Abraham, R., Golberg, L., and Coulston, F. Uptake and storage of degraded carrageenan in lysosomes of reticuloendothelial cells of the Rhesus monkey, *Macaca Mulatta*. *Exp Mol Pathol*, **17**: 77-93, 1972.

Arakawa, S., Ito, M., and Tejima, S. Promoter function of carrageenan on development of colonic tumors induced by 1,2-dimethylhydrazine in rats. *J Nutr Sci Vitaminol*, **34**: 577-585, 1988.

Benitz, K. F., Golberg, L., and Coulston, F. Intestinal effects of carrageenans in the Rhesus monkey. *Fd Cosmet Toxicol* **11**: 565-575, 1973.

Carlucci, M.J., Ciancia, M., Matulewicz, M.C., Cerezo, A.S., and Damonte, E.B. Antitherpetic activity and mode of action of natural carrageenans of diverse structural types. *Antiviral Res*, **43**: 93-102, 1999.

Carthew, P. Safety of Carrageenan in Foods. *Correspondence, Environmental Health Perspectives*, **110**: 2002.

Coggins, C., Blanchard, K., Alvarez, F., Brache, V., Weisberg, E., Kilmarx, P.H., Lacarra, M., Massai, R., Mishell, Jr., D., Salvatierra, A., Witwatwongwana, P., Elias, C., and Ellertson, C. Preliminary safety and acceptability of a carrageenan gel for possible use as a vaginal microbicide. *Sex Transm Inf*, **76**: 480-483, 2000.

Cohen, B. I., Raicht, R. F., Deschner, E. E., Takahashi, M. and Sarwal, A. N. Effect of cholic acid feeding on N-methyl-N-nitrosourea-induced colon tumors and cell kinetics in rats. *J Natl Cancer Inst*, **64**, 573-578, 1980.

Cohen, S. M., and Ito, N. A critical review of the toxicological effects of carrageenan and processed eucheuma seaweed on the gastrointestinal tract. *Crit Rev Toxicology*, **32**: 413-444, 2002.

Cook, H.A., Williams, D., and Tomas, G.A. Crypt-restricted metallothionein immunopositivity in murine colon: validation of a model for studies of somatic stem cell mutation. *J Pathol*, **191**: 306-312, 2000.

Corpet, D. E., Taché, S., and Préclaire, M. Carrageenan given as a jelly, does not initiate, but promotes the growth of aberrant crypt foci in the rat colon. *Cancer Lett* **114**: 53-55, 1997.

Coste, M., Dubuquoy, C., and Tome´, D. Effect of systemic and orally administered ioto-carrageenan on ovalbumin-specific antibody response in the rat. *Int Arch Allergy Appl Immunol*, **88**: 474-476, 1989.

Donnelly, E. T., Bardwell, H., Thomas, G. A., Williams, E. D., Hoper, M., Crowe, P., McCluggage, W. G., Stevenson, M., Phillips, D. H., Hewer, A., Osborne, M. R. and Campbell, F. C. Modulation of N-methyl-N-nitrosourea-induced crypt restricted metallothionein immunopositivity in mouse colon by a non-genotoxic diet-related chemical. *Carcinogenesis* **25**: 847-855, 2004a.

Donnelly, E. T., Bardwell, H., Thomas, G. A., Williams, E., Hoper, M., Crowe, P., McCluggage, W. G., Phillips, D. H., Osborne, M., Hewer, A., Stevenson, M., and Campbell, F. Colonic crypt stem cell mutation indices (CCSCMI) in a predictive risk assessment model for diet related chemicals. *Gastroenterology*, **126**: A505, 2004b.

Donnelly, E. T., Bardwell, H., Thomas, G. A., Williams, E. D., Hoper, M., Crowe, P., McCluggage, W. G., Stevenson, M., Phillips, D. H., Hewer, A., Osborne, M., and Campbell, F. C. Dietary interactions and inception of colonic tumourigenesis. *Gut*, **53**: A121, 2004c.

Donnelly, E. T., Bardwell, H., Thomas, G. A., Sillwyn, W. E., Williams, E. D., Hoper, M., Crowe, P., McCluggage, W. G., Stevenson, M., Phillips, D. H., Hewer, A., Osborne, M. R. and Campbell, F. C. Metallothionein crypt-restricted immunopositivity indices (MTCRII) correlate with aberrant crypt foci (ACF) in mouse colon. *Br J Cancer*, **92**: 2160-2165, 2005.

Engster, M., and Abraham, R. Cecal response to different molecular weights and types of carrageenan in the guinea pig. *Toxicol Appl Pharmacol* **38**: 265-282, 1976.

Frossard, C.P., Hauser, C., and Eigenmann, P.A. Oral carrageenan induces antigen-dependent oral tolerance: prevention of Anaphylaxis and induction of lymphocyte anergy in a murine model of food allergy. *Pediatr Res* **49**: 417-422, 2001.

Hagiwara, A., Miyashia, K., Nakanishi, T., Sano, M., Tamano, S., Asai, I., Nakamura, M., Imaida, K., Ito, N., and Shirai, T. Lack of tumor promoting effects of carrageenan on 1,2-dimethylhydrazine-induced colorectal carcinogenesis in male F344 rats. *J Toxicol Pathol* **14**: 37-43, 2001.

Haijin, M., Xiaolu, J., and Huashi, G. A  $\gamma$ -carrageenan derived oligosaccharide prepared by enzymatic degradation containing anti-tumor activity. *J Appl Phycology*, **15**: 297-303, 2003.

Hicks, R. M., Wakefield, J. S. J., and Chowaniec, J. Evaluation of a new model to detect bladder carcinogens or co-carcinogens; Results obtained with saccharin, cyclamate and cyclophosphamide. *Chemico-Biological Interactions*, **11**: 225-233, 1975.

IARC (International Agency for Research on Cancer). Carrageenan *IARC Monograph*, **32**: 79-94, 1983.

International Food Additives Council and Marinalg International. Carrageenan Monograph, 1983 (unpublished).

International Food Additives Council and Marinalg International/CLITAM. Carrageenan Monograph, 1991 (unpublished).

International Food Additives Council and Marinalg International/CLITAM. Carrageenan Monograph, 1997 (unpublished).

Jasani, B., Campbell, F., Navabi, H., Schmid, K.W., and Williams, G.T. Clonal overexpression of metallothionein is induced by somatic mutation in morphologically normal colonic mucosa. *JPathol*, **184**: 144-147, 1998.

JECFA: Joint FAO/WHO Expert Committee on Food Additives. Toxicological evaluation of certain food additives, including anticaking agents, antimicrobials, antioxidants,

emulsifiers and thickening agents. World Health Organization Technical Report Series, Forty-two Report, 1999, *FAO Nutr Report Series*, 1999.

JECFA: Safety evaluation of certain food additives and contaminants. Prepared by the Fifty-seventh meeting of the Joint FAO/Who Expert Committee on Food Additives (JECFA). Carrageenan and Processed Eucheuma Seaweed (addendum). Who Food Additives Series. **48**: 91-101, 2002.

B. Koletzko *et al.* Global standard for the Composition of Infant Formula: Recommendations of an ESPGHAN coordinated international expert group. *J Pediatric Gastroenterology and Nutrition*, **41**: 584-599, 20

Lambrecht, V., Boilly, B. and LeBourhis, X. Regulation of cell proliferation and urokinase plasminogen activation of human breast epithelial cells by carrageenan. *Int J Oncology*, **12**: 1397-1401, 1998.

Li, Y., Mao, Wenjun. Characterization of carrageenan derived oligosaccharides and its effect on T-cell function and subset in irradiated mice. Abstracts of papers, 228<sup>th</sup> ACS Nat'l mtg. , Phila., PA. *Am. Chem. Soc.* 2004

Liu, J.M., Haroun-Bouhedja, F. and Boisson-Vidal, C. Analysis of the in vivo inhibition of mammary adenocarcinoma cell adhesion by sulphated polysaccharides. *Anticancer Res*, **20**: 3265-3271, 2000.

Melnick, R. L., Huff, J., Haseman, J.K., et al. chronic effects of agar, guar gum, gum arabic, locust-bean gum, or tara gum in F344 rats and B6C3F1 mice. *Food Chem Toxicol*, **21**: 305-311, 1983.

Mikalsen, S. and Sanner, T. Intercellular communication in colonies of Syrian hamster embryo cells and the susceptibility for morphological transformation. *Carcinogenesis*, **14**: 251-257, 1993.

Munro, I. C., and Danielewska-Nikiel, B. Comparison of estimated daily intakes of flavouring substances with no-observed-effect levels. *Food Chem Toxicol*, **44**: 758-809, 2006.

Nacife, C.P., Soeiro, M., and Gomes, R. Morphological and biochemical characterization of macrophages activated by carrageenan and lipopolysaccharide in vivo. *Cell Structure Function*, **29**: 27-34, 2004.

Nagoaka, M., Shibata, H., Kimura-Takagi, I., Hashimoto, S., Aiyama, R., Ueyama, S., and Yokokura, T. Anti-ulcer effects and biological activities of polysaccharides from marine algae. *BioFactors*, **12**: 267-274, 2000.

Narisawa, T., Fukaura, Y., and Takeba, N., and Nakai, K. Chemoprevention of N-methylnitrosourea-induced colon carcinogenesis by ursodeoxycholic acid-5-aminosalicylic acid conjugate in F344 rats. *Jpn J Cancer Res*, **93**: 143-150, 2002.

Panlasigui, L.N., Baello, O.Q., Dimatangal, J.M., and Dumelod, B.D. Blood cholesterol and lipid-lowering effects of carrageenan on human volunteers. *Asia Pacific J Clin Nutr*, **12**: 209-214, 2003

Papanikolaou, A., Wang, Q. S., Papanikolaou, D., Whiteley, H. E., and Rosenberg, D. W. Sequential and morphological analysis of aberrant crypt foci formation in mice of differing susceptibility to azoxymethane-induced colon carcinogenesis. *Carcinogenesis*, **21**: 1567-1572, 2000.

Rivedal, E., Yamasaki, H., and Sanner, T. Inhibition of gap junctional intercellular communication in Syrian hamster embryo cells by TPA, retinoic acid and DDT. *Carcinogenesis*, **15**: 689-694, 1994.

Rosen, P.P., and Oberman, H.A. Tumors of the Mammary Gland. *Atlas of Tumor Pathology*, Third Series Fascicle 7, 1993.

Ruch, R. J., Klaunig, J. E., and Pereira, M. A. Inhibition of intercellular communication between mouse hepatocytes by tumor promoters. *Toxicol Appl Pharmacol*, **87**: 111-120, 1987

Rutten, A. A. J. J. L., Jongen, W. M. F., de Haan, L. H. J., Hendriksen, E. G. J., and Koeman, J. H. Effect of retinol and cigarette-smoke condensate on dye-coupled intercellular communication between hamster tracheal epithelial cells. *Carcinogenesis*, **9**: 315-320, 1988.

Suzuki, J., Na, H., Upham, B.L., Chang, C. and Trosko, J.E.  $\beta$ -carrageenan-induced inhibition of gap-junctional intercellular communication in rat liver epithelial cells. *Nutrition Cancer*, **36**: 122-128, 2000.

Taché, S., Peiffer, G., Millet, A. and Corpet, D. E. Carrageenan Gel and Aberrant Crypt Foci in the Colon of Conventional and Human Flora-Associated Rats. *Nutrition Cancer*, **37**: 193-198, 2000.

Tarlo, Susan M., Dolovich, J., and Listgarent, C. Anaphylaxis to carrageenan: A pseudo-latex allergy. *J Allergy Clin Immunol*, **95**: 933-936, 1995.

Tobacman, J.K. Filament disassembly and loss of mammary myoepithelial cells after exposure to ( $\lambda$ ) carrageenan. *Cancer Research*, **37**: 2823-2826, 1997

Tobacman, J.K. Carrageenan exposure leads to mammary myoepithelial cell development of unusual intracellular membranes. *Proc American Assoc Cancer Res*, **40**: 715, 1999.

Tobacman, J.K. Review of harmful gastrointestinal effects of carrageenan in animal experiments. *Environ Health Perspect*, **109**: 983-994, 2001.

Tobacman, J.K. Carrageenan in foods: response. *Environ Health Perspect*, **110**: A176-A177, 2002.

Tobacman, J.K. Toxic considerations related to ingestion of carrageenan. *Reviews in Food and Nutrition Toxicity*, **1**: 204-229, 2003.

Tobacman, J.K. and Walters, K.S. Carrageenan-induced inclusions in mammary myoepithelial cells. *Cancer Detect Prev*, **25**: 520-526, 2001.

Tobacman, J.K. and Zhila, K. Reduced expression of steroid sulfatase in mammary myoepithelial cells following exposure to lambda-carrageenan. *Proc Amer Assoc Cancer Research*, **43**: 1079, 2002.

Tobacman, J.K. Wallace, R.B. and Zimmerman, B. Association between consumption of carrageenan and other gums used as food additives and incidence of mammary carcinoma in the US during the twentieth century. *Proc Amer Assoc Cancer Research*, **41**: 92, 2000.

Tobacman, J.K. Wallace, R.B. and Zimmerman, B. Consumption carrageenan and water-soluble polymers used as food additives and incidence of mammary carcinoma. *Medical Hypothesis*, **56**: 589-598, 2001.

Tobacman, J.K. Wallace, R.B., Stumbo, P., and Nicols, S. Dietary carrageenan content estimated from Iowa Women's Health Study food-frequency questionnaires. *Cancer Epidemiol Biomarkers Prevention*, **11**: 1211, 2002.

Tsuji, R.F., Hosino, K., Noro, Y., Tsuji, N.M., Masuda, T., Akira, S., and Nowak, B. Suppression of allergic reaction by  $\lambda$ -carrageenan: Toll-like receptor 4/MyD88-

dependent and –independent modulation of immunity. *Clin Exp Allergy*, **33**: 249-258, 2003.

United States Adopted Names (USAN) Council. List No. 297, New Names-Poligeenan. *Clin Pharmacol Ther*, **44**: 246-248, 1988.

Weiner, M. L. Intestinal transport of some macromolecules in food. *Fd Chem Toxicol*, **26**: 867-880, 1988.

Weiner, M. L. Toxicological properties of carrageenan. *Agents Actions*, **32**: 46-51, 1991.

Weiner, M. L., Nuber, D., Blakemore, W. R., Harriman, J. F. and Cohen, S. M. A 90-day Dietary Study of Kappa Carrageenan with Emphasis on the Gastrointestinal Tract. *Food Chem Toxicol*, In Press, 2006.

West, R.W., W.G. Sheldon, Gaylor, D.W., Allen, R.R., Kadlubar, F. F. Study of sodium saccharin co-carcinogenicity in the rat. *Food Chem Toxicol* **32**: 207-213, 1994.

Willett, W. C., Hunter, D. J., Stampfer, M. J., Colditz, G., Manson, J. E., Spiegelman, D., Rosner, B., Hennekens, C. H., Speizer, F. E. Dietary fat and fiber in relation to risk of breast cancer. *J Amer Med Assoc*, **268**: 2037-2044, 1992

Yamada, T., Ogamo, A., Saito, T., Watanabe, J., Uchiyama, H., and Nakagawa, Y. Preparation and anti-HIV activity of low-molecular-weight carrageenans and their sulfated derivatives. *Carbohydr Polym*, **32**: 51-55, 1997.

Yuan, H. and Song, J. Preparation, structural characterization *in vitro* antitumor activity of kappa-carrageenan oligosaccharide fraction from *Kappaphycus striatum*. *J Applied Physio*, **17**: 7-13, 2005.

Zhou, G., Sun, Y., Xin, H., Zhang, Y., Li, Z., and Xu, Z. In vivo antitumor and immunomodulation activities of different molecular weight lambda-carrageenans from *Chondrus ocellatus*. *Pharmacol Res*, **50**: 47-53, 2004

Zhou, G., Xin, H., Sheng, W., Sun, Y., Li, Z., and Xu, Z. In vivo growth-inhibition of S180 tumor by mixture of 5-Fu and low molecular  $\lambda$ -carrageenan from *Chondrus ocellatus*. *Pharmacol Res*, **51**: 153-157, 2005.

Zhou, G., Sheng, W., Yao, W., Wang, C. Effect of low molecular lambda-carrageenans from *Chondrus ocellatus* on antitumor H-22 activity of 5-FU. *Pharmacol Res*, **53**: 129-34, 2006.

## **Part 3. Safety assessment for infants 0 – 6 months from exposure to carrageenan through infant formulas**

### **3.1 Discussion**

Some concerns have been raised regarding possible greater sensitivity to gastrointestinal effects of carrageenan in infants compared to adolescents or adults (Koletzko et al, 2005)<sup>1</sup>. This appears to be based on a report of a reaction to a barium enema as well as reports in the literature of colitis arising in experimental systems following the administration of carrageenan. The basis for both of these phenomena seems to be incorrectly derived.

To begin with, the barium enema very likely was composed of poligeenan rather than carrageenan. Critiques of the details of this study are included elsewhere in this document (see Section 2.7 and Appendix 3).

The issue of colitis following administration of carrageenan is somewhat controversial, but is likely related to differences in composition of the administered material and the dose at which it was administered. In the study by Weiner et al (2006), using food grade carrageenan and utilizing an extensive examination of the gastrointestinal tract of rats in a GLP study, there was no evidence of colitis. Other reports suggesting colitis utilized carrageenan that was not food grade, most likely of much higher viscosity and at very high exposure levels, or involved administration of poligeenan. Studies with poligeenan are not relevant to the risk assessment of carrageenan. There is some evidence in guinea pigs of absorption of carrageenan across the gastrointestinal tract mucosa with some evidence of colitis, but this appears

to be specific to the guinea pig and does not involve systemic exposure, as there is no evidence of excretion in the urine.

A critical evaluation of the literature strongly suggests that colitis does not occur following administration of food grade carrageenan either to experiment animals or to humans.

A possible increased sensitivity of infants to possible colitis induction by carrageenan administration also requires some evaluation. As just indicated, it is highly unlikely that adolescents or adults will develop colitis following oral consumption of food grade carrageenan. More importantly, there is no evidence that undigestible polysaccharides, such as other fibers and including carrageenan, will produce colitis in infants. If anything, infants should actually have a decreased susceptibility to any potential for colitis given the more rapid transit time through the gastrointestinal tract when fibers are present in the diet at relatively high levels. In addition, this more rapid transit secondary to high levels of undigestible polysaccharides might have an influence on the bacterial flora, but evidence suggests that this does not affect the induction of colitis or other adverse effects in the infant other than the possibility of mild and transient diarrhea. There is diarrhea only if there is a high level of undigestible polysaccharide content of the diet compared to low levels. Carrageenan is likely to only be present at relatively low levels in humans, including infants, so is unlikely to have any effect on transit time or flora.

A key to an evaluation of carrageenan with respect to this evaluation is that it is relevant to carrageenan, which is a very large molecular weight, undigestible polysaccharide. It is not an evaluation of poligeenan, which is much smaller in molecular weight and has a potential for hydrolysis and a potential for inducing some inflammatory changes in the gastrointestinal tract.

---

<sup>1</sup> The ESPHAGN coordinated group provided recommendations to the CCFNSDU on the global standard for the composition of infant formula. It appears that this body did not take into consideration the 1998 and 2001 JECFA

Based on this discussion, like adults, it is highly unlikely that there will be an inflammatory reaction in infants, and if anything, there should be less susceptibility to gastrointestinal inflammatory effects of carrageenan than in the adolescent or adult.

### **3.2 References<sup>2</sup>**

B. Koletzko *et al.* Global standard for the Composition of Infant Formula: Recommendations of an ESPGHAN coordinated international expert group. *J Pediatric Gastroenterology and Nutrition*, **41**: 584-599, 2005.

---

reviews of carrageenan as these were not referenced in the published report.

<sup>2</sup> See Section 2.9 References

## **Overall Conclusions**

- The current specifications as written are satisfactory to assure suitable quality of carrageenan and Processed Eucheuma Seaweed for human consumption.
- Current information supports the consolidation of carrageenan INS 407 and Processed Eucheuma Seaweed INS 407a as one food additive to be called carrageenan INS 407.
- Poligeenan is a separate and distinct chemical substance that is distinguishable from carrageenan.
- Carrageenan and Processed Eucheuma Seaweed remain additives that are obtained by well defined production methods and remain stable throughout manufacture when utilized in production
- The recent 90 day rat feeding study dispels concerns about whether there is toxicological significance to the lower molecular weight tail of carrageenan thereby confirming and expanding upon knowledge of carrageenan's safety.
- Carrageenan remains a safe and suitable food additive for use in formula for infants.

## **Appendices**

## Appendix 1

### PROPOSED SPECIFICATIONS FOR CARRAGEENAN DEFINED AS THE CONSOLIDATION OF INS 407 CARRAGEENAN AND INS 407a PROCESSED EUCHEUMA SEAWEED

|            |  |
|------------|--|
| SYNONYMS   | Irish moss (from <i>Chondrus</i> spp.); Eucheuman (from <i>Eucheuma</i> spp.); Iridophycan (from <i>Iridaea</i> spp.); Hypnean (from <i>Hypnea</i> spp.); Furcelleran or Danish agar (from <i>Furcellaria fastigiata</i> ); Processed Eucheuma seaweed, PES, PNG-carrageenan, semi-refined carrageenan.  |
| DEFINITION | <p>A substance with hydrocolloid properties obtained from certain members of the class Rhodophyceae (red seaweeds).</p> <p>The principal commercial sources of Carrageenans are the following families and genera of the class of Rhodophyceae:<br/>Furcellariaceae such as <i>Furcellaria</i><br/>Gigartinales such as <i>Chondrus</i>, <i>Gigartina</i>, <i>Iridaea</i><br/>Hypnaceae such as <i>Hypnea</i><br/>Phyllophoraceae such as <i>Phyllophora</i>, <i>Gymnogongrus</i>, <i>Ahnfeltia</i><br/>Solieriaceae such as <i>Eucheuma</i>, <i>Anatheca</i>, <i>Meristotheca</i>.</p> <p>Carrageenan is a hydrocolloid consisting mainly of the ammonium, calcium, magnesium, potassium, and sodium sulfate esters of galactose and 3,6-anhydrogalactose polysaccharides. These hexoses are alternately linked <math>\alpha</math>-1,3 and <math>\beta</math>-1,4 in the copolymer. The relative proportions of cations existing in carrageenan may be changed during processing to the extent that one may become predominant.</p> <p>The prevalent polysaccharides in carrageenan are designated as kappa-, iota-, and lambda-carrageenan. Kappa-carrageenan is mostly the alternating polymer D-galactose-4-sulfate and 3,6-anhydro-D-galactose; iota-carrageenan is similar, except that the 3,6-anhydrogalactose is sulfated at carbon 2. Between kappa-carrageenan and iota-carrageenan there is a continuum of intermediate compositions differing in degree of sulfation at carbon 2. In lambda-carrageenan, the alternating monomeric units are</p> |

mostly D—galactose-2-sulfate (1,3-linked) and D-galactose-2,6-disulfate (1,4-linked).

Carrageenan is obtained in a number of ways. It may be extracted from seaweed into water or aqueous dilute alkali. Carrageenan may be recovered by alcohol precipitation, by drum drying, or by precipitation in aqueous potassium chloride and subsequent freezing. It may also be processed by soaking the cleaned seaweed in alkali for a short time at elevated temperatures. The material is then thoroughly washed with water to remove residual salts followed by purification, drying, and milling to a powder. The alcohols used during recovery and purification are restricted to methanol, ethanol, and isopropanol.

Articles of commerce may include sugars for standardized purposes, salts to obtain specific gelling or thickening characteristics, or emulsifiers carried over from drum drying processes.

DESCRIPTION Yellow or tan to white, coarse to fine powder.

FUNCTIONAL USES Thickener, gelling agent, stabilizer, emulsifier.

| PURITY         | CARRAGEENAN (INS 407) – CURRENT             | PROCESSED EUCHEUMA SEAWEED (INS 407a) – CURRENT | CONSOLIDATED CARRAGEENAN AND PROCESSED EUCHEUMA SEAWEED – PROPOSED NAME – CARRAGEENAN; PROPOSED INS – 407 |
|----------------|---|---|---|
| Loss on drying | Not more than 12% (105° to constant weight) | Not more than 12% (105° to constant weight)     | Not more than 12% (105° to constant weight)   |
| pH             | Between 8 and 11 (1 in 100 suspension)      | Between 8 and 11 (1 in 100 suspension)          | Between 8 and 11 (1 in 100 suspension)  |

|                                   |  |  |  |
|-----------------------------------|--|--|--|
| Viscosity                         | Not less than 5 cp at 75° (1.5% solution). See description under TESTS   | Not less than 5 cp at 75° (1.5% solution). See description under TESTS   | Not less than 5 cp at 75° (1.5% solution). See description under TESTS   |
| Sulfate                           | Not less than 15% and not more than 40% (as SO <sub>4</sub> <sup>2-</sup> ) on a dry weight basis. See description under TESTS.  | Not less than 15% and not more than 40% (as SO <sub>4</sub> <sup>2-</sup> ) on a dry weight basis. See description under TESTS.  | Not less than 15% and not more than 40% (as SO <sub>4</sub> <sup>2-</sup> ) on a dry weight basis. See description under TESTS.  |
| Total Ash                         | Not less than 15% and not more than 40%  | Not less than 15% and not more than 30% on a dry weight basis. See description under TESTS.  | Not less than 15% and not more than 40%  |
| Acid-insoluble ash                | Not more than 1%   | Not more than 1%.  | Not more than 1%.  |
| Acid insoluble matter             | Not more than 2%. Use 2 g of sample obtained from part (a) of the procedure for sulfate determination.   | Not less than 8% and not more than 15% on a dry weight basis. Use 2 g of sample obtained from part (a) of the procedure for sulfate determination.   | Not more than 15%.   |
| Residual solvents                 | Not more than 0.1% of ethanol, isopropanol, or methanol singly or in combination. See description under TESTS  | Not more than 0.1% of ethanol, isopropanol, or methanol singly or in combination. See description under TESTS  | Not more than 0.1% of ethanol, isopropanol, or methanol singly or in combination. See description under TESTS  |
| Microbiological criteria (Vol. 4) | Initially prepare a 10 <sup>-1</sup> dilution by adding a 50 g sample to 450 m. of Butterfield's phosphate – buffered dilution water and homogenizing the mixture in a high speed blender. | Initially prepare a 10 <sup>-1</sup> dilution by adding a 50 g sample to 450 m. of Butterfield's phosphate –buffered dilution water and homogenizing the mixture in a high speed blender. Total aerobic plate count: Not more than | Initially prepare a 10 <sup>-1</sup> dilution by adding a 50 g sample to 450 m. of Butterfield's phosphate – buffered dilution water and homogenizing the mixture in a high speed blender. |

|                 |   |  |   |
|-----------------|---|--|---|
|                 | Total aerobic plate count: Not more than 5000 cfu/g.<br>Salmonella spp.: Negative per test.<br>E. coli: Negative in 1 g.  | 5000 cfu/g.<br>Salmonella spp.: Negative per test.<br>E. coli: Negative in 1 g.  | Total aerobic plate count: Not more than 5000 cfu/g.<br>Salmonella sop.: Negative per test.<br>E. coli: Negative in 1 g   |
| Arsenic (Vol 4) | Not more than 3 mg/kg   | Not more than 3 mg/kg  | Not more than 3 mg/kg   |
| Lead (Vol 4)    | Not more than 5 mg/kg. Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods." | Not more than 5 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods." | Not more than 5 mg/kg. Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods." |
| Cadmium         | Not more than 2 mg/kg. See description under TESTS.   | Not more than 2 mg/kg. See description under TESTS.  | Not more than 2 mg/kg. See description under TESTS.   |
| Mercury         | Not more than 1 mg/kg. See description under TESTS.   | Not more than 1 mg/kg. See description under TESTS.  | Not more than 1 mg/kg. See description under TESTS.   |
|                 |   |  |   |
|                 |   |  |   |

## **Appendix 2**

### **PROPOSED INS NUMBER FOR CARRAGEENAN DEFINED AS THE CONSOLIDATION OF CARRAGEENAN INS 407 AND PROCESSED EUCHEUMA SEAWEED INS 407a**

Presently, carrageenan and processed eucheuma seaweed are treated as separate and distinct food additives, each being assigned a unique INS number 407 and 407a respectively. The assignment of separate INS numbers and specifications was appropriate at the time that processed eucheuma seaweed entered the market. Over the years industry has gained a better understanding of the physical/chemical aspects of PES, its toxicology and its applications to food. The quality of PES has increased thereby more closely aligning it with carrageenan. Further, in many applications the two products can be and are used interchangeably.

In this context, the NGO Marinalg International proposes that PES no longer be distinguished from carrageenan by the separate INS 407a. Marinalg International proposes that PES be included under the INS 407 and henceforth be called carrageenan.

### Appendix 3

#### **Poligeenan – A Single Purpose Pharmaceutical Product**

##### Poligeenan Structure

Poligeenan is the name given to the product made by the deliberate acid-hydrolysis (at pH below 1.5, temperatures above 80<sup>0</sup>C, and times over 1h) of sulfated polygalactose of the same backbone composition as carrageenan. The molecular weight of poligeenan is in the range 10,000 to 20,000 daltons. This is far below the molecular weight range of 100,000 to 150,000 daltons previously mentioned as corresponding to the minimum water viscosity specification of 5 cps (1.5% at 75C) for the food additive carrageenan. The water viscosity of poligeenan under these conditions measures about 0.1 cps. Poligeenan would need to be tested at about 5% concentration to achieve 5 cps viscosity.

Poligeenan has none of the attributes or functionalities described for carrageenan or PES used in food. It does not gel, and, at best, has very poor thickening and stabilizing qualities.

There has been much confusion in the literature between “carrageenan” and “poligeenan” as much of the earlier work referred to this product as “degraded carrageenan”. This confusion is dealt with in detail in the toxicology section of this monograph.

The initial application of poligeenan in the late 1960s was for the temporary relief of peptic ulcer symptoms. The poligeenan lined and thus protected the stomach wall from gastric acid. It also functioned as an anti-histamine with histamine believed at the time to be involved in the formation of peptic ulcers. Low volumes of poligeenan are still used for this application.

However, the primary current application for poligeenan is as an excipient in barium enemas for enhanced X-ray diagnostic of the upper GI tract. These products comprise 25-85% barium sulphate (w/w) and have the consistency of a milk shake. There are four primary valued attributes of the poligeenan: (a) it provides a high charge density to prevent the barium sulphate from “packing”, (b) it provides the rheological consistency of a drinkable shake, (c) it does not gel, and (d) it has the cold solubility and dispersability to function as a dry-mix. “Packing” is the formation of associated barium sulphate particles that will not re-suspend on shaking. These are key functional criteria as the barium enema application includes both ready-to-drink and dry-mix products in addition to the wide range of barium sulphate content already detailed above.

The low molecular weight of poligeenan is key and essential to meet all these requirements. Carrageenan simply cannot meet all four requirements for application in barium enemas, as the target charge density is not compatible with either the acceptable rheology (including non-gelation) or the necessary dispersability / solubility. For example, the viscosity of a 5% solution of poligeenan is about 5cps, but a 5% solution of non-gelling carrageenan ranges from about 100 to 5,000 cps over the Mw range 200,000 to 800,000 Daltons. Carrageenan at the 5% level would also fail the dry-mix dispersability / solubility requirement. In addition, commercial kappa and iota carrageenans gel at concentrations above about 0.1%. Also, for the same viscosity measurement, the charge density of commercial carrageenan (200,000 to 800,000 Daltons) would only be one fiftieth to one five hundredth that of poligeenan.

So, carrageenan and poligeenan are two distinct products targeted at two distinct markets. They are not interchangeable in any way whatsoever. Blends of poligeenan and carrageenan make no commercial sense. Carrageenan adds nothing to the poligeenan application for the reasons given above, and there is no application where adding the much more expensive poligeenan to carrageenan would serve any functional or economic benefit to food grade carrageenan. Further complicating the situation is the slow acceptance outside the US of the “poligeenan” nomenclature per the Tarlo study (Tarlo ).

