CARRAGEENAN

EXECUTIVE SUMMARY

REJECT relisting of carrageenan on the National List, and **reject any proposed annotation**, for the following reasons:

- Scientists have warned for decades that carrageenan in foods is **harmful to human health**, because of a preponderance of scientific evidence linking carrageenan to **gastrointestinal inflammation and colon cancer**.

- Degraded carrageenan is listed as a "**possible human carcinogen**" by the WHO's International Agency for Research on Cancer.

- Industry data from 2005 show that the industry has no reliable way of testing levels of degraded carrageenan in their products. **All tested samples of food-grade carrageenan contained varying levels of potentially carcinogenic degraded carrageenan.**

- Research indicates food-grade carrageenan breaks down in the digestive tract, and becomes degraded in the human body.

CARRAGEENAN

Based on current scientific research, carrageenan is not a safe ingredient in food and should be removed from the National List.

The scientific community has recognized for decades that degraded carrageenan is harmful to human health. The carrageenan industry will therefore likely suggest an annotation, prohibiting only degraded carrageenan from organic foods. Degraded carrageenan has low molecular weight, and has been recognized since the 1960s as causing gastrointestinal inflammation. Degraded carrageenan has been listed since 1983 as a "possible human carcinogen" by the WHO's International Agency for Research on Cancer.

An annotation of this nature would have been appropriate in the mid-1990s, when this material was originally reviewed, because data did not yet exist to counter the claim that food-grade carrageenan can be entirely free of degraded carrageenan. In the mid-1990s, therefore, it would have been reasonable to assume that degraded carrageenan could be isolated in the marketplace, and prohibited in organics—but the Technical Advisory Panel (TAP) reviewers did not suggest any annotation.

But industry data from 2005 (submitted as an attachment) has revealed that varying levels of degraded carrageenan contaminate all food-grade carrageenan. Moreover, studies have shown harmful health effects of food-grade carrageenan,

and scientists suggest that carrageenan in fact degrades in the human digestive system.

The option of an annotation would therefore be virtually meaningless and should be rejected, since industry data show clearly that **no food-grade carrageenan can claim to be safe and free from degraded carrageenan**.

It is important to note that the Joint FAO/WHO Expert Committee on Food Additives determined in 2001 that food-grade carrageenan should contain less than 5% degraded carrageenan, since carrageenan is listed as a "possible human carcinogen" by the WHO's International Agency for Research on Cancer. In response, Marinalg, the trade group for carrageenan manufacturers, set out to determine if they could meet this requirement. They concluded they could not reliably determine the levels of degraded carrageenan in their products.

Results from the 2005 Marinalg Working Group's tests (page 2, Figure 1 – submitted as an attachment) clearly show that degraded carrageenan, a substance that is known to cause colon inflammation and is classified by the WHO's International Agency for Research on Cancer as a "possible human carcinogen," was present in all samples of food-grade carrageenan. Therefore, all carrageenan should be prohibited from foods, especially organic foods.

This information was publicly available before the last sunset review. If a Technical Review had been requested by the NOSB, which it was not, and if it was independently produced and judiciously executed, the Board would have been able to weigh these concerns five years ago.

The current Technical Review on carrageenan points out negative human health and environmental impacts. In an attempt at balance, the TR mistakenly lists a study by Tobacman and Walters (line 582) as contradicting findings of gastrointestinal tract inflammation. This study does not address inflammation and should therefore not have been listed as contradicting findings of human health concerns.

The only study included in the TR that contradicts findings of gastrointestinal tract inflammation is authored by ML Weiner, who, at the time of the study, was employed by FMC Corporation, one of the major producers of carrageenan.

In this attempt at "balance," the anonymous authors of the TR fail to point out that **the vast preponderance of peer-reviewed, published scientific literature on this matter clearly indicates a threat to human health**.

Please find attached our full review of the scientific literature and our analysis of carrageenan's safety.

Based on scientific research and industry data, the inclusion of carrageenan on the National List is illegal, since it violates the Organic Foods Production Act of 1990,

Sec. 2118(c)(1)(A)(i) - "the National List may provide for the use of substances only if ... the use of such substances would not be harmful to human health or the environment."

Carrageenan fails the following criterion in the organic standards: 7 CFR 205.600(b)(3) - "The nutritional quality of the food is maintained when the substance is used, and the substance, itself, **or its breakdown products** do not have an adverse effect on human health as defined by applicable Federal regulations."

Please note that the standards include the phrase "or its breakdown products." Research shows that food-grade carrageenan is broken down in the gastrointestinal tract to degraded carrageenan, a "possible human carcinogen." Food-grade carrageenan should therefore be removed from the National List based on 7 CFR 205.600(b)(3).

It is now clear that the NOSB approval process of carrageenan in the mid-1990s, including the review by a Technical Advisory Panel, was seriously flawed. The three TAP reviewers failed to raise known concerns about carrageenan, despite a number of published peer-reviewed articles in and letters to scientific journals pointing out health concerns. **Scientists have published letters in journals such as *The Lancet* as early as 1980 pointing out human health concerns with food-grade carrageenan**.

Please also note that a petition is pending with the FDA to remove carrageenan's GRAS (Generally Recognized As Safe) status. The FDA never performed a review of carrageenan's safety, since the ingredient's GRAS approval was "grandfathered in."

Organic foods should be held to a higher standard of safety. The FDA has not acted to remove carrageenan from foods, and **the Board must show consumers that the organic industry is serious about providing a safe alternative to the conventional food supply**. We urge the Board to remove all carrageenan from the National List, and reject any attempts by carrageenan manufacturers and theircustomers to keep carrageenan on the list with a meaningless annotation.

CORNUCOPIA
INSTITUTE

Food-Grade Carrageenan:
Reviewing Potential Harmful Effects on Human Health

Executive Summary

Carrageenan is derived from red seaweed, and is used as an ingredient in foods such as dairy, dairy alternatives (such as soy-based beverages and desserts) and deli meats as a thickening agent, stabilizer and/or emulsifier.

Carrageenan can be classified as low molecular weight, "degraded" carrageenan, or high molecular weight, or "undegraded" carrageenan.

Degraded, low molecular weight carrageenan is recognized as a carcinogen in lab animals, and is therefore classified as a "possible human carcinogen" by the International Agency for Research on Cancer.[1]

Degraded carrageenan also causes inflammation in the colon in rodents, which resembles ulcerative colitis, an inflammatory bowel disease.[2] This inflammatory property of degraded carrageenan is not in dispute, especially since the medical research community has used degraded carrageenan for decades to induce acute inflammation in experimental trials conducted with lab animals, to test anti-inflammation drugs.[3] [4] [5] [6] [7]

Carrageenan processors tend to portray the difference between degraded and undegraded carrageenan as a simple, black-and-white distinction. They claim that food-grade carrageenan sold to food processors falls entirely in the undegraded category.

However, studies (including industry-funded studies) show that food-grade carrageenan is also linked to colon inflammation and colon cancer in animals. Studies have reported that high molecular weight carrageenan can degrade in the gastrointestinal tract to low molecular weight carrageenan.[8, 9]

Moreover, when the industry tested its food-grade carrageenan for the presence of degraded carrageenan, results showed that every sample had at least some degraded carrageenan, with some test results of food-grade carrageenan showing as much as 25% degraded carrageenan.

Timeline

1960's -present: Starting in 1961, animal studies consistently show that degraded carrageenan is carcinogenic.[10] [11] [12] [13] [14]

1969: Researchers find that degraded carrageenan causes ulcerations and inflammation in lab animals that closely resemble ulcerative colitis, a human inflammatory bowel disease.[15]

1969 - present: Researchers testing treatments for ulcerative colitis use degraded carrageenan to induce the disease in laboratory animals.[16] [17] [18] [19]

1973: A study shows that degraded carrageenan induces inflammation in the digestive system of monkeys. This shows that degraded carrageenan affects the gastrointestinal system of primates as well as rodents.[20]

1975: A study with rhesus monkeys finds adverse effects in the intestinal tract when the animals were given low levels (1% solution) of **undegraded** carrageenan in their drinking water.[21]

1978: A study published in *Cancer Research* finds that rats fed a diet containing **undegraded** carrageenan had higher rates of cancer than rats fed a control diet without carrageenan. The authors conclude: "**The undegraded carrageenan in the diet had an enhancing effect in colorectal carcinogenesis in rats**."[22]

1980-1981: Leading carrageenan researchers R. Marcus and James Watt publish two letters in the *Lancet*, titled "Danger of Carrageenan in Foods" and "Potential Hazards of Carrageenan," pointing out health concerns with the consumption of carrageenan, **including undegraded carrageenan**.

They note that the harmful effects of undegraded carrageenan in animals "are almost certainly associated with its degradation during passage through the gastrointestinal tract."[23]

1983: With adequate scientific data showing the carcinogenicity of degraded carrageenan in lab animals, the International Agency for Research on Cancer (IARC) classifies **degraded** carrageenan as Group 2B, "*Possibly carcinogenic to humans*."[24] The Agency determines that there is **not enough evidence to classify undegraded carrageenan** as a possible human carcinogen.

1986: A study finds that exposure of rats to 6% **undegraded** carrageenan in the diet for 24 weeks, with weekly injections of the carcinogenic substance 1,2-dimethylhydrazine (1,2-DMH), was associated with an **increase in tumors** from 40% to 75% and with the more frequent occurrence of larger and proximal tumors.[25]

1995: Three scientists perform the Technical Advisory Panel (TAP) review[26] for the National Organic Standards Board, to determine whether carrageenan is an ingredient appropriate for use in organic foods. None of the three reviewers mentions the carcinogenicity in animal studies of degraded carrageenan, or the "possibly carcinogenic to humans" classification by the IARC. None mention the studies suggesting possible adverse health effects of undegraded carrageenan.

One reviewer downplays the potential human health effects of carrageenan by writing: "Carrageenan has a high molecular weight and must be distinguished from lower molecular weight "degraded" carrageenan which may have adverse health effects."

The reviewers doing the 1995 TAP review do not include more recent studies (widely available in 1995) pointing to potential human health problems, such as the 1992 study by Wilcox et al, with Proctor and Gamble, that finds an association between epithelial cell loss and the consumption of both undegraded and degraded carrageenan.[27]

1996: The National Research Council of the National Academy of Science adopts the IARC classification for degraded carrageenan (possible human carcinogen).[28]

2001: A study finds higher levels of tumors in rats given food-grade carrageenan, yet reports that the difference is not statistically significant. This study, partially funded by the food industry, publishes its findings with the conclusive and misleading title and conclusion that food-grade, "undegraded" carrageenan is safe (despite its findings of higher cancer rates). Marinalg, the industry trade group for carrageenan processors, uses the study to reassure its customers that carrageenan is safe.[29]

June 2001: A Joint FAO/WHO Expert Committee on Food Additives (JECFA) recommends an Acceptable Daily Intake of "not specified" for carrageenan. Marinalg hails the decision and claims it confirms the safety of carrageenan.[30]

September 2001: Joanne Tobacman, MD, then Assistant Professor of Clinical Medicine at the University of Iowa (now Associate Professor of Clinical Medicine at the University of Illinois at Chicago), publishes an article in the academic, peer-reviewed journal *Environmental Health Perspectives*. Dr. Tobacman conducted an independent review of the scientific literature on carrageenan, and concluded: "Because of the acknowledged carcinogenic properties of degraded carrageenan in animal models **and the cancer-promoting effects of undegraded carrageenan in experimental models**, the widespread use of carrageenan in the Western diet should be reconsidered" (emphasis added).[31]

March 2003: The European Commission's Scientific Committee on Food reviews Tobacman's 2001 article, and reviews recent safety data on carrageenan. The Committee suggests that the amount of degraded carrageenan in food-grade

carrageenan be kept to levels below 5%, "in order to ensure that the presence of any degraded carrageenan is kept to a minimum."[33]

The Commission also reaffirms its earlier position that it remains inadvisable to use carrageenan as an ingredient in infant formula.

2005: Marinalg, the industry trade group, convenes a working group to determine the levels of degraded carrageenan in its products.[34] The working group tests 12 samples of food-grade carrageenan from a variety of suppliers in six different laboratories, to measure the presence of degraded carrageenan and determine if the 5% limit is feasible.

The results from the industry's own test results are cause for serious concern. First, the levels of degraded carrageenan detected in the samples varied considerably depending on the laboratory performing the tests. This suggests that even the industry does not have a reliable way of determining the levels of degraded carrageenan in food-grade carrageenan.[35] If the carrageenan manufacturers have no reliable way of testing levels of degraded carrageenan in their products, how can they claim their food-grade carrageenan is safe?

Second, the results showed that 8 of the 12 samples of food-grade carrageenan contained higher than 5% degraded carrageenan according to at least one of the laboratories (in many cases, according to multiple laboratories).

Most alarmingly, **all samples contained at least some degraded carrageenan according to the majority of laboratories**.

Not a single sample could confidently claim to be entirely free of the material that is classified as a "possible human carcinogen."

The highest level of degraded carrageenan found in a sample was 25%.

2002-2012: Industry-sponsored scientists question whether the inflammatory nature of carrageenan is rodent-specific, and whether the results of animal studies can be extrapolated to humans.[36] [37] Scientists conduct experiments using human colonic epithelial cells and find that carrageenan, even low levels of food-grade carrageenan, induce inflammation in human colon cells as well.[38] [39] [40] [41]

2008: The National Organic Standards Board considers whether to re-allow carrageenan during the Sunset process. No public interest groups or scientists chime in. The NOSB receives ten comments from industry, including carrageenan manufacturers, the Organic Trade Association, and various organic food manufacturers using carrageenan, all claiming carrageenan is safe and essential in organic processing.[42]

January 2012: Marinalg reports that, after eight years of planning, experimentation, and analysis (2003 to 2011), the industry has been unable to reliably measure the levels of degraded carrageenan in its products in the laboratories of its members, its customers, or in independent laboratories.[43]

May 2012: The National Organic Standards Board again reviews carrageenan during the Sunset process, and will decide whether to continue allowing carrageenan in certified organic foods.

Q&A: Essentiality in Organic Handling

Q: Are there alternatives to carrageenan for food processors?

A: Yes. On supermarket shelves, equivalent organic products appear side-by-side with some containing carrageenan and others without carrageenan. Food processors can use organic gums, including organic guar gum and organic locust bean gum as alternatives to carrageenan.

Q: Do other gums used as stabilizers and thickening agents raise the same health concerns?

A: No. Carrageenan, unlike other gums, is highly sulfated and contains certain bonds (alpha-1,3-disaccharide bonds) which are foreign to human cells and stimulate an innate immune response. This immune response leads to inflammation, which can be chronic. In the intestine, chronic inflammation is associated with the development of malignant cancer.

Q: Is carrageenan essential in organic handling?

A: No. For every organic product on store shelves containing carrageenan, an equivalent product by another manufacturer can be found that does not contain carrageenan.

Soy Creamer: Wildwood Pulmuone "soy creamer" contains carrageenan, whereas Organic Valley "soy creamer" does not.

Aseptic Soy Milk: Silk unsweetened soymilk contains carrageenan, whereas Eden Foods unsweetened soymilk does not.

Refrigerated Soy Milk: Earth Balance and Silk contain carrageenan, whereas Organic Valley does not.

Low Fat Cottage Cheese: Horizon lowfat cottage cheese contains carrageenan whereas Organic Valley low fat cottage cheese uses organic guar gum and organic locust bean gum instead.

And more…. The marketplace has already shown that acceptable alternatives to carrageenan exist.

Summary

The Organic Foods Production Act of 1990 (OFPA) sought to establish an alternative food system for consumers wishing to avoid potentially dangerous substances in the food supply.

OFPA allows up to 5% (by weight) non-organic ingredients in a processed organic food, but only if "the use of such substances **would not be harmful to human health** or the environment" (OFPA Sec. 2118(c)(1)(A)(i)).

The USDA's organic standards require the following of non-organic, non-agricultural substances allowed in organic foods: "The substance itself, **or its breakdown products**, do not have adverse effects on human health as defined by applicable Federal regulations" (emphasis added) (7 CFR 205.600(b)(3)).

The organic standards include the requirement that the substance's "breakdown products" do not have adverse effects on human health, and industry data show that food-grade carrageenan contains levels of carcinogenic degraded carrageenan, sometimes as high as 25%. **Moreover, research shows that food-grade carrageenan can be broken down to degraded carrageenan in the gastrointestinal tract.**

Scientific evidence shows that the consumption of food-grade carrageenan may lead to harmful effects on human health, including inflammation, lesions, and cancer in the colon.

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[1] http://monographs.iarc.fr/ENG/Monographs/vol31/volume31.pdf

[2] Delahunty T, Recher L, Hollander D.. Intestinal permeability changes in rodents: a possible mechanism for degraded carrageenan-induced colitis. Food Chem Toxicol 25:113–118. 1987.

[3] IARCIARC Working Group on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Carrageenan. IARC Monogr Eval Carcinog Risk Hum 31:79–94. 1983.

[4] Nicklin S, Miller K.. Effect of orally administered food-grade carrageenans on antibody-mediated and cell-mediated immunity in the inbred rat. Food Chem Toxicol 22:615–621. 1984.

[5] Thomson AW, Fowler EF. Carrageenan: a review of its effect on the immune system. Agents Actions 1:265–273. 1981.

[6] Salyers AA, West SHE, Vercelotti JR, Wilkins TD. Fermentation of mucins and plant polysacchairds by anerobic bacteria from the human colon. Appl Environ Microbiol 334:529–533. 1977.

[7] Di Rosa M.. Review: Biological properties of carrageenan. J Pharm Pharmacol 24:89–102. 1972

[8] Pittman KA, Golberg L, Coulston F. Carrageenan: the effect of molecular weight and polymer type on its uptake, excretion and degradation in animals. Food Cosmet Toxicol 14:85–93. 1976.

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

[10] Cater DB. The carcinogenic action of carrageenin in rats. Br J Cancer 15:607–614. 1961

[11] Ashi KW, Inagaki T, Fujimoto Y, Fukuda Y. Induction of degraded carrageenan of colorectal tumors in rats. Cancer Lett 4(3): 171-6. 1978.

[12] Mankes R, Abraham R. Lysosomal dysfunction in colonic submucosal macrophages of rhesus monkeys caused by degraded iota carrageenan. Proc Soc Exp Biol Med. 1975 Oct;150(1):166–170.

[13] Rustia M, Shubik P, Patil K.. Lifespan carcinogenicity tests with native carrageenan in rats and hamsters. Cancer Lett 11:1–10. 1980.

[14] Hopkins J.. Carcinogenicity of carrageenan. Food Cosmet Toxicol 19:779–788. 1981.

[15] Watt J, Marcus R.. Ulcerative colitis in the guinea-pig caused by seaweed extract. J Pharm Pharmacol 21:187S–188S. 1969

[16] Kitsukawa Y, Saito H, Suzuki Y, Kasanuki J, Tamura Y, Yoshida S.. Effect of ingestion of eicosapentaenoic acid ethyl ester on carrageenan-induced colitis in guinea pigs. Gastroenterology 102:1859–1866. 1992

[17] Jensen BH, Andersen JO, Poulsen SS, Olsen PS, Rasmussen SN, Hansen SH, Hvidberg DF. The prophylactic effect of 5-aminosalicylic acid and salazosulphapyridine on degraded-carrageenan-induced colitis in guinea pigs. Scand J Gastroenterol 19:299–303. 1984

[18] Watt J, Marcus SN, Marcus AJ. The comparative prophylactic effects of sulfasalazine, prednisolone, and azathioprine in experimental ulceration. J Pharm Pharmacol 32:873–874. 1980.

[19] Kitano A, Matsumoto T, Oshitani N, Nakagawa M, Yasuda K, Watanabe Y, Tomobuchi M, Obayashi M, Tabata A, Fukushima R, et al. Distribution and anti-inflammatory effect of mesalazine on carrageenan-induced colitis in the rabbit. Clin Exp Pharmacol Physiol 23:305–309. 1996.

[20] Benitz K-F, Golberg L, Coulston F. Intestinal effects of carrageenans in the rhesus monkey (Macaca mulatta). Food Cosmet Toxicol 11:565–575 (1973)

[21] Mankes R, Abraham R. Lysosomal dysfunction in colonic submucosal macrophages of rhesus monkeys caused by degraded iota carrageenan. Proc Soc Exp Biol Med. 1975 Oct;150(1):166–170.

[22] Watanabe K, Reddy BS, Wong CQ, Weisburger JH. Effect of dietary undegraded carrageenan on colon carcinogenesis in F344 rats treated with azoxymethane or methylnitrosourea. Cancer Res 38:4427–4430. 1978.

[23] Marcus R, Watt J.. Danger of carrageenan in foods and Lancet 1:338. 1981, Marcus R, Watt J.. Potential hazards of carrageenan Lancet 1:602–603. 1980

[24] http://monographs.iarc.fr/ENG/Monographs/vol31/volume31.pdf

For undegraded (native) carrageenan, the IARC noted the following: "In female rats treated with azoxymethane or Nnitrosomethylurea together with **native** carrageenan in the diet, a **greater incidence of colorectal cancers** was observed than with treatment by azoxymethane or N-nitrosomethylurea alone." Yet despite this finding, the IARC classified undegraded carrageenan as "Group 3," "*Not classifiable as to its carcinogenicity to humans*." Note that this is different from the classification of "Group 4," which is "*Probably not carcinogenic to humans*"

[25] Arakawa S, Okumua M, Yamada S, Ito M, Tejima S.. Enhancing effect of carrageenan on the induction of rat colonic tumors by 1,2-dimethylhydrazine and its relation to ß-glucuronidase activities in feces and other tissues. J Nutr Sci Vitaminol (Tokyo) 32:481–485. 1986.

[26] TAP Review on Carrageenan by Dr. Richard Theuer. Available online at http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5067875&acct=nopgeninfo. Last accessed April 4, 2012.

[27] Wilcox DK, Higgins J, Bertram TA. Colonic epithelial cell proliferation in a rat model of nongenotoxin-induced colonic neoplasia. Lab Invest 67:405–411. 1992.

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[29] Hagiwara A, Miyashita 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 Vol. 14; 37. (2001)

[30] http://www.marinalg.org/PDF/1_Safety_of_carrageenan_and_processed_euchema_seaweed.pdf

[31] Tobacman JK. Review of Harmful Gastrointestinal Effects of Carrageenan in Animal Experiments. Environ Health Perspect 109(10). 2001

[33] http://ec.europa.eu/food/fs/sc/scf/out164_en.pdf

[34] Status report on the work of Marinalg International to measure the molecular weight distribution of carrageenan and PES in order to meet the EU specification: less than 5% below 50,000 Daltons. Marinalg. Available online at: http://www.marinalg.org/PDF/_FULL_Molecular_weight_distribution_of_carrageenan_and_PES.pdf. Last accessed April 4, 2012

[35] In an earlier version of the Working Group's report, Marinalg admitted: "At the time of writing (November, 2005) the Working Group has not found a method for molecular weight distribution measurement that is sufficiently accurate and reproducible to yield a validated and defensible method."

[36] Cohen SM and Ito M. A Critical Review of the Toxicological Effects of Carrageenan and Processed Eucheuma Seaweed on the Gastrointestinal Tract. Crit. Rev. Toxicol. 32(5): 413-444. 2002.

The paper is sponsored in part by Marinalg, the industry trade group for carrageenan processors.

[37] Carthew P. Safety of Carrageenan in Foods. Environ Health Perspect 110:a176-a176. 2002.

Carthew, at the time of writing this correspondence, is an employee of Unilever.

[38] Bhattaycharyya S, Borthakur A, Dudeja PK, Tobacman JK. Carrageenan induces interleukin-8 production through distinct Bcl10 pathway in normal human colonic epithelial cells. Am J Physiol Gastrointest Liver Physiol 2007;292(3):G829-38. Epub 2006 Nov 9.

[39] Bhattacharyya S, Dudeja PK, Tobacman JK. Tumor necrosis factor alpha-induced inflammation is increased but apoptosis is inhibited by common food additive carrageenan. J Biol Chem. 2010 Dec 10;285(50):39511-22. Epub 2010 Oct 11

[40] Bhattacharyya S, Gill R, Chen M-L, Zhang F, Linhardt RJ, Dudeja PK, Tobacman JK. Toll-like receptor 4 mediates induction of Bcl10-NFκB-IL-8 inflammatory pathway by carrageenan in human intestinal epithelial cells. J Biol Chem.2008;283(16):10550-8. E pub 2008 Feb 5

[41] Bhattacharyya S, Borthakur A, Tyagi S, Gill R, Chen ML, Dudeja PK, Tobacman JK. B-cell CLL/lymphoma 10 (BCL10) is required for NF-kappaB production by both canonical and noncanonical pathways and for NF-kappaB-inducing kinase (NIK) phosphorylation .J Biol Chem. 2010 Jan 1;285(1):522-30. Epub 2009 Nov 6

[42] Although The Cornucopia Institute was in its fourth year of operation in 2008, our primary focus was farm policy. As we have grown and matured, and it has become obvious that there is a need for independent scrutiny of materials petitions and sunset reviews, we are committing greater resources to providing balance and oversight of the organic materials review process at the USDA.

[43] "Status report on the work of Marinalg International to measure the molecular weight distribution of carrageenan and PES in order to meet the EU specification: less than 5% below 50,000 daltons." Available at www.marinalg.org, last accessed April 3, 2012.

TECHNICAL POSITION ON MEASUREMENTS RELATED TO MEETING THE EC MOLECULAR WEIGHT DISTRIBUTION SPECIFICATION FOR CARRAGEENAN AND PES

The Marinalg Working Group on Molecular Weight Determination (William Blakemore FMC, Chairman; Dr. Harris Bixler, SIAP, Secretary; Arne Graff Anderson, CPKelco; Dr. Joop de Vries, Danisco; Dr. Patrick Boulenguer, Degussa) has been carrying out experiments since April, 2003 to measure the molecular weight distribution of commercial carrageenan and PES used in foods. It was on March 5, 2003 that the EC-SCF expressed an opinion proposing a new specification for these hydrocolloids to augment the 5 cps water viscosity "*if feasible"*.

The purpose of the new specification is to have better control over the amount of very low molecular weight carrageenan and PES going into food products. Oligomers of carrageenan less than 10,000 daltons (Da) in molecular weight have a history of causing adverse toxicological effects when fed in large quantities to certain rodents; although there is no epidemiological evidence that the very small amounts of these oligomers that might be present in carrageenan or PES being used in foods have caused any harmful effects to humans.

Before the Marinalg Working Group of carrageenan producers had adequate time to determine the feasibility of measuring the new specification, it was formally adopted by the EC as Commission Directive 2004/45/EC on April 16, 2004 for implementation by Member States by April 1, 2005. This specification requires that carrageenan or PES used in food must not contain more than 5% molar mass with molecular weight less than 50,000 Da. To save space in this document, this low molecular weight tail will be abbreviated as the LMT.

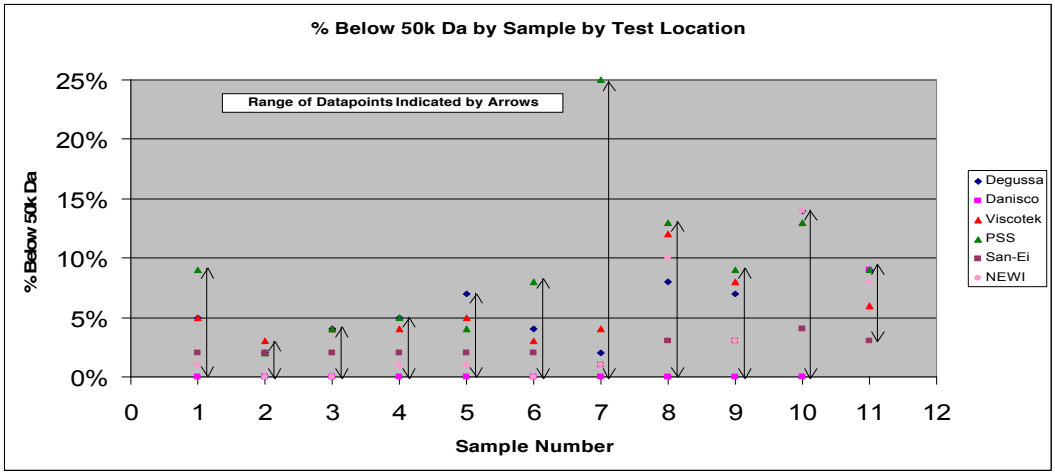
At the time of writing (November, 2005) the Working Group has not found a method for molecular weight distribution measurement that is sufficiently accurate and reproducible to yield a validated and defensible method.

The methods studied have all been based on size exclusion chromatography (SEC) followed by concentration and molecular weight detection in the stream exiting the chromatography columns. SEC is used to spread out the carrageenan molecular size distribution in the flow stream exiting the columns. Note that this separation is by molecular size and not molecular weight, so physical models are used to convert molecular size data to molecular weights. The stream exiting the SEC columns flows through a series of detectors: refractive index for carrageenan concentration determination and light scattering and/or intrinsic viscosity for molecular weight determination. Some instruments include chemical detectors to be sure only carrageenan is being measured in the flow stream.

These are highly developed commercial research instruments of great technical sophistication. Nevertheless, none met the most important objective of the

Working Group. Six laboratories participated in this study, Degussa; Danisco; Viscotek, Ltd; Polymer Standards Services, GmbH; San-Ei Gen FFI, Ltd: North East Wales Institute/NEWI, all with state-of-the art equipment and with qualified scientists to run the experiments. Procedure details (sample preparation and concentration, eluent type and concentration, etc.) were recorded for each lab and approved by the Working Group. Eleven different commercial carrageenan and PES samples, representing different sulfated polygalactose (nominally kappa, lambda and iota) made by five different producers, were tested by all laboratories under "Round Robin" conditions. Annex 1 (the Summary Sheet and the Sample Information Sheet) contains test results and physical characteristics of the Round Robin samples, respectively.

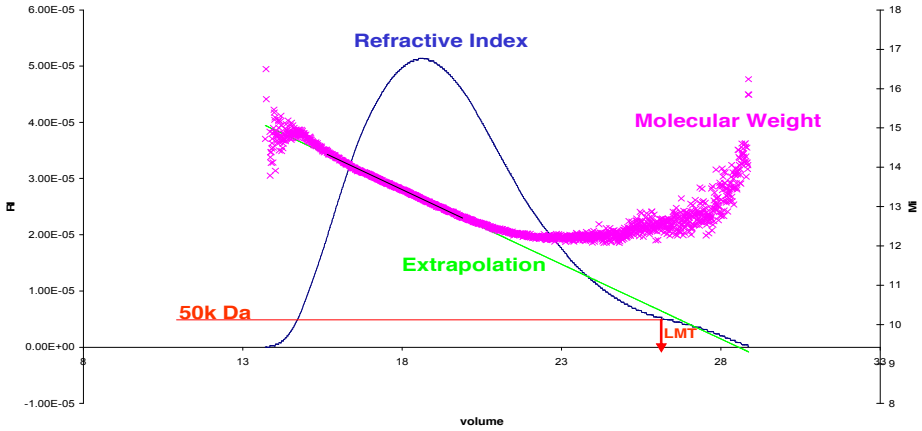
Figure 1



Despite all this technical discipline, inter-lab reproducibility of the LMT was shown to be poor (Fig. 1). (Readers desiring larger format figures for more careful study of results are referred to Annex II.). Detectors downstream of the SEC columns must be able to measure polymer concentration and molecular weight accurately in the range represented by the LMT. It appears that even under optimum SEC conditions, detector signal to noise ratio (S/N) in the LMT region is extremely low, especially for light scattering upon which molecular weight determination is based (Fig. 2). Several test locations have experienced drifting baselines, and variable recoveries (measured concentration versus actual), both of which make data interpretation even more complex and unreliable. Initially the Working Group thought that the Viscotek triple detector method (refractive index, low angle light scattering and intrinsic viscosity) was giving promising results, and as a consequence published the method on the Marinalg website. However, further testing indicated that these same issues applied, but to a lesser degree.

Figure 2

Typical output from SEC/RI/MALLS - Degussa Data



Various physical models of molecular weight determination by light scattering (Zimm, Debye, Berry) are being used in SEC / light scattering software to extrapolate from a region of the distribution with good S/N into the region of poor S/N. For all of the carrageenan samples studied the S/N within the LMT was low and resulted in extrapolations having to be made from well outside the LMT range (Fig. 2, green line). This type of extrapolation is subject to enough error so as not to give defensible results for regulatory purposes. This can be seen in Fig. 2 where the LMT region is shown graphically. Clearly any shift in baseline or green line extrapolation will have a profound effect on the very small LMT region calculated for commercial carrageenan being used in foods. It is estimated that it is virtually impossible to determine the molecular weight of SEC-spread samples below about 10,000 Da by any of the light scattering techniques.

The Working Group's experience with SEC/light scattering in no way detracts from it use as a valuable research tool. The technique is widely used for estimating polymer molecular structure in food and industrial applications. A higher level of accuracy, however, is required when it is to be used for specification and regulatory measurements. Even in the present study valuable information (from the Round Robin samples) was obtained. For instance, fairly good consistency was seen for inter-lab results obtained for the weight average molecular weight (Mw) (Fig. 3), except for one sample, a lambda type that is known to be a more rigid rod in solution that the kappa and iota types. Furthermore there was fairly good correlation for Mw versus water viscosity (Fig. 3), except for the one aberrant sample already noted. However, when looking at the LMT data, both these consistencies and correlations were generally poor (Fig. 4)

Figure 3

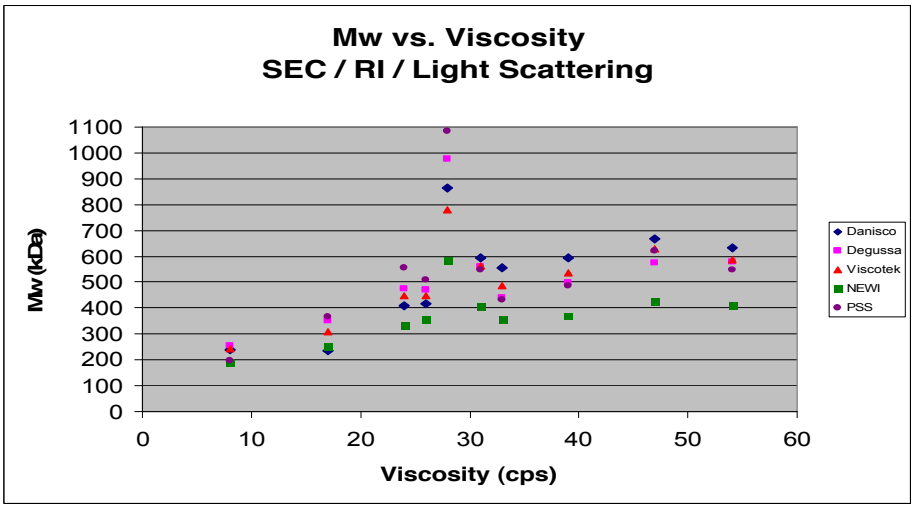
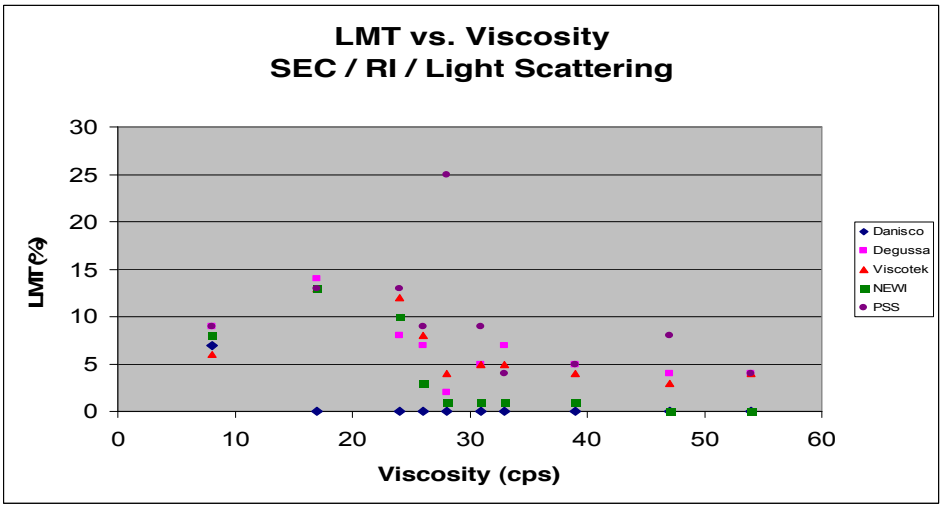


Figure 4



The Working Group has conferred with several world class scientists (Prof. Wayne Reed, Tulane University, Dr. Phillip Wyatt and his staff at Wyatt Technology, and the group consisting of Drs. Chi-San Wu, E. Malawer and L. Senak at ISP and Dr. Maguarite Rinaudo at CMRV) who have been involved in developing and using SEC/light scattering for a variety of research purposes. While some were confident that the Working Group's goal could be reached,

none had ever done so. Through this process consensus was gradually reached that the current equipment employing light scattering and the attendant software will not measure the EC specification with sufficient accuracy to survive the necessary validation protocols.

While the work to date with light scattering has led to frustrating conclusions, it has pointed in a direction of potentially more promise which will be explored by the Working Group. Light scattering became dominant in the measurement of polymer molecular weight distributions because molecular weights exiting a SEC column over most of a samples' range (except the LMT) could be determined directly. Prior to this advancement, column calibration with molecular weight standards had to be used.

This technique involves preparing a calibration curve of exit time from the SEC column versus molecular weight for a set of standards of very narrow molecular weight distribution (Mw/Mn < 1.2) (polydispersity index or PDI). The molecular weight of the polymer standards is now usually determined by light scattering. No SEC is required when the molecular weights are being determined, and sample concentrations can be adjusted to optimize the S/N ratio. The polymer standards must encompass the molecular weight range of interest for samples being used in a SEC study. For water soluble hydrocolloids, the most widely used standards are eight pullulans ranging in Mw from 5,300 to 760,000 Daltons that are commercially available from Shodex. This method has been tested on commercial carrageenans, and the results have been reported in the scientific literature by Japanese scientists (Uno, et al, Food Additives and Contaminants, *18*, No. 9, pp763-772, 2001). No correction was applied in this work for the differences between pullulan and carrageenan sizes versus molecular weights, so validation of the LMTs reported by Uno remains in question.

Of course, having a set of carrageenan standards would be preferable and the Working Group is exploring the preparation of such a set. It should be noted, however, that producing carrageenan standards with PDI < 1.2 will be very difficult, and from past experience PDI values would be expected to be 1.6 at best and more likely closer to 2.0, probably outside the range of PDI needed for LMT accuracy.

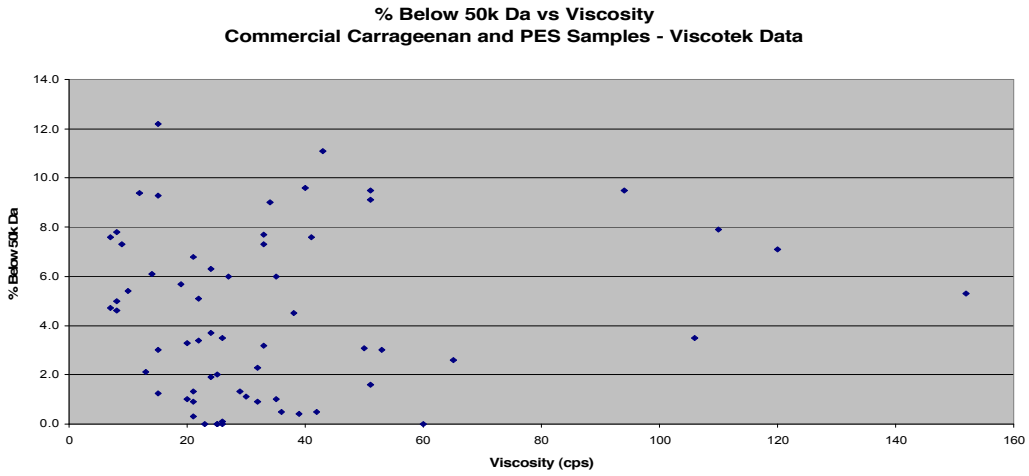
The difficulties in obtaining the Standards has lead the Working Group to explore the application of a technology referred to as "universal calibration", a physical model for converting a pullulan calibration curve to a carrageenan calibration curve (Grubisic, Z. et al, Polymer Letters, *5*, pp753-759, 1967). The model takes into consideration size and shape differences for the two polymers when their molecular weights are the same. Initial work applying this technique to the Uno data shows some promise, but it is too early to draw any conclusions.

A related technology referred to as "polydisperse or broad standard calibration" is also under investigation (Malawer, E.G. and A.J. Montana, Journal of Polymer Science: Polymer Physics Edition, *18*, pp2303-2305, 1980). For this purpose, a very broad molecular weight distribution carrageenan is prepared as a standard

that has relatively high concentrations of carrageenan in the low and high molecular weight tails and spans the range of Mw of interest. Again, physical modeling and computer analysis is employed to convert SEC exit time to a carrageenan molecular weight.

There is no assurance until experiments can be run to know whether to poor accuracy of LMT calculation from light scattering can be improved upon by use of either universal or polydisperse calibration.

Figure 5



The Working Group also went back to the JECFA and FCC water viscosity method of specifying a pseudo molecular weight limit on carrageenan and PES to see if it could be improved upon to identify commercial products with a satisfactory LMT. Fig. 5 shows that products of nearly identical water viscosity can have very different LMTs, at least to the qualitative degree to which light scattering LMT measurements can be relied upon. Note however, that although there is no correlation between the LMT and viscosity, the correlation between molecular weight (Mw) remains fairly good as described earlier (Fig. 3).

It is clear at this point in time that successfully measuring the EC specification is not currently feasible. The Marinalg Working Group will continue to explore methods, but cannot guarantee success.

Of greater significance to human safety are the results of a recent 90-day rat feeding study that was performed with a carrageenan very close to the 5 cps water viscosity limit (8cps). The study showed no adverse toxicological effects in the test animals. A poster presentation of this work, delivered at the Society of Toxicology 2004 annual meeting, is contained on the Marinalg Website, and a complete article that is to appear in 2006 in a peer reviewed journal is pending.

This very important feeding study is more thoroughly summarized in an introduction and synopsis to this technical position paper. It was prepared by members of Marinalg's Technical and Regulatory Committee and appears as a position paper on the Marinalg website through the following link <xxxx>. It should satisfy even the most concerned reader that failing to measure the new EC specification results in no increased risk to human health.

END