June 7, 2008

Dockets Management Branch
Food and Drug Administration
Department of Health and Human Services, Room 1-23
12420 Parklawn Dr.
Rockville, MD 20857

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CITIZEN PETITION

The undersigned submits this petition under the Federal Food, Drug, and Cosmetic Act, the Public Health Service Act, or any other statutory provision for which authority has been delegated to the Commissioner of Food and Drugs (under 21 CFR, Part 5.10) to request the Commissioner of Food and Drugs to **revoke** regulations that support the direct addition of carrageenan or carrageenan salts or furcelleran or furcelleran salts to food for human consumption.

A. Action Requested

The action requested is to revoke the status of the food additive carrageenan and carrageenan salts as a food additive permitted for direct addition to food for human consumption, as indicated in 172.620 and 172.626 of Title 21, CFR, Chapter 1, Subchapter B, as cited below. **Carrageenans should not be permitted for direct addition to food for human consumption and its use should be prohibited. The inclusion of carrageenan or carrageenan salts in other food products or in other uses in which carrageenan is consumed by humans, as specified in other paragraphs, should be prohibited. In addition, furcelleran and furcelleran salts should not be permitted for direct addition to food for human consumption.** Regulations pertinent to furcelleran are 172.655 and 172.660 of Title 21, CFR, Chapter 1, Subchapter B. Since furcelleran is structurally similar to carrageenan, findings pertinent to carrageenan and carrageenan salts are also pertinent to furcelleran and furcelleran salts.

TITLE 21—FOOD AND DRUGS
CHAPTER I--FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH AND HUMAN SERVICES
SUBCHAPTER B--FOOD FOR HUMAN CONSUMPTION
(CONTINUED)

PART 172 -- FOOD ADDITIVES PERMITTED FOR DIRECT ADDITION TO FOOD FOR HUMAN CONSUMPTION

Subpart G--Gums, Chewing Gum Bases and Related Substances

Sec. 172.620 Carrageenan.

The food additive **carrageenan** may be safely used in food in accordance with the following prescribed conditions:

FDA.2008.P.0347.0001

CP

(a) The food additive is the refined hydrocolloid prepared by aqueous extraction from the following members of the families Gigartinaceae and Solieriaceae of the class Rodophyceae (red seaweed):

Chondrus crispus.

Chondrus ocellatus.

Eucheuma cottonii.

Eucheuma spinosum.

Gigartina acicularis.

Gigartina pistillata.

Gigartina radula.

Gigartina stellata.

(b) The food additive conforms to the following conditions:

(1) It is a sulfated polysaccharide the dominant hexose units of which are galactose and anhydrogalactose.

(2) Range of sulfate content: 20 percent to 40 percent on a dry-weight basis.

(c) The food additive is used or intended for use in the amount necessary for an emulsifier, stabilizer, or thickener in foods, except for those standardized foods that do not provide for such use.

(d) To assure safe use of the additive, the label and labeling of the additive shall bear the name of the additive, carrageenan.

Database Updated April 1, 2007

TITLE 21--FOOD AND DRUGS
CHAPTER I--FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH AND HUMAN SERVICES
SUBCHAPTER B--FOOD FOR HUMAN CONSUMPTION
(CONTINUED)

PART 172 -- FOOD ADDITIVES PERMITTED FOR DIRECT ADDITION TO FOOD FOR HUMAN CONSUMPTION

Subpart G--Gums, Chewing Gum Bases and Related Substances

Sec. 172.626 Salts of carrageenan.

The food additive salts of **carrageenan** may be safely used in food in accordance with the following prescribed conditions:

(a) The food additive consists of carrageenan, meeting the provisions of 172.620, modified by increasing the concentration of one of the naturally occurring salts (ammonium, calcium, potassium, or sodium) of **carrageenan** to the level that it is the dominant salt in the additive.

(b) The food additive is used or intended for use in the amount necessary for an emulsifier, stabilizer, or thickener in foods, except for those standardized foods that do not provide for such use.

(c) To assure safe use of the additive, the label and labeling of the additive shall bear the name of the salt of **carrageenan** that dominates the mixture by reason of the modification, e.g., "sodium carrageenan", "potassium carrageenan", etc.

Database Updated April 1, 2007

133: CHEESES AND RELATED CHEESE PRODUCTS
§ 133.178 - Pasteurized neufchatel cheese spread with other foods.
§ 133.179 - Pasteurized process cheese spread.

136: BAKERY PRODUCTS
§ 136.110 - Bread, rolls, and buns.

139: MACARONI AND NOODLE PRODUCTS
§ 139.121 - Nonfat milk macaroni products.
§ 139.122 - Enriched nonfat milk macaroni products.

150: FRUIT BUTTERS, JELLIES, PRESERVES, AND RELATED PRODUCTS

§ 150.141 - Artificially sweetened fruit jelly.
§ 150.161 - Artificially sweetened fruit preserves and jams.

172: FOOD ADDITIVES PERMITTED FOR DIRECT ADDITION TO FOOD FOR HUMAN CONSUMPTION
§ 172.620 - Carrageenan.
§ 172.623 - Carrageenan with polysorbate 80.
§ 172.626 - Salts of carrageenan.

176: INDIRECT FOOD ADDITIVES: PAPER AND PAPERBOARD COMPONENTS
§ 176.170 - Components of paper and paperboard in contact with aqueous and fatty foods.

201: LABELING
§ 201.319 - Water-soluble gums, hydrophilic gums, and hydrophilic mucilloids (including, but not limited to agar, alginic acid, calcium polycarbophil, carboxymethylcellulose sodium, carrageenan, chondrus, glucomannan ((B-1,4 linked) polymannose acetate), guar gum, karaya gum, kelp, methylcellulose, plantago seed (psyllium), polycarbophil tragacanth, and xanthan gum) as active ingredients; required warnings and directions.

310: NEW DRUGS
§ 310.545 - Drug products containing certain active ingredients offered over-the-counter (OTC) for certain uses.

TITLE 21--FOOD AND DRUGS
CHAPTER I--FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH AND HUMAN SERVICES
SUBCHAPTER B--FOOD FOR HUMAN CONSUMPTION (CONTINUED)

PART 172 -- FOOD ADDITIVES PERMITTED FOR DIRECT ADDITION TO FOOD FOR HUMAN CONSUMPTION

Subpart G--Gums, Chewing Gum Bases and Related Substances

Sec. 172.655 Furcelleran.

The food additive **furcelleran** may be safely used in food in accordance with the following prescribed conditions:

(a) The food additive is the refined hydrocolloid prepared by aqueous extraction of furcellaria fastigiata of the class Rodophyceae (red seaweed).

(b) The food additive conforms to the following:

(1) It is a sulfated polysaccharide the dominant hexose units of which are galactose and anhydrogalactose.

(2) Range of sulfate content: 8 percent to 19 percent, on a dry-weight basis.

(c) The food additive is used or intended for use in the amount necessary for an emulsifier, stabilizer, or thickener in foods, except for those standardized foods that do not provide for such use.

(d) To assure safe use of the additive, the label and labeling of the additive shall bear the name of the additive, furcelleran.

TITLE 21--FOOD AND DRUGS
CHAPTER I--FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH AND HUMAN SERVICES
SUBCHAPTER B--FOOD FOR HUMAN CONSUMPTION (CONTINUED)

PART 172 -- FOOD ADDITIVES PERMITTED FOR DIRECT ADDITION TO FOOD FOR HUMAN CONSUMPTION

Subpart G--Gums, Chewing Gum Bases and Related Substances

Sec. 172.660 Salts of furcelleran.

The food additive salts of **furcelleran** may be safely used in food in accordance with the following prescribed conditions:

(a) The food additive consists of furcelleran, meeting the provisions of 172.655, modified by increasing the concentration of one of the naturally occurring salts (ammonium, calcium, potassium, or sodium) of **furcelleran** to the level that it is the dominant salt in the additive.

(b) The food additive is used or intended for use in the amount necessary for an emulsifier, stabilizer, or thickener in foods, except for those standardized foods that do not provide for such use.

(c) To assure safe use of the additive, the label and labeling of the additive shall bear the name of the salt of **furcelleran** that dominates the mixture by reason of the modification, e.g., "sodium furcelleran", "potassium furcelleran", etc.

B. Statement of Grounds

The grounds on which this petition for revocation of carrageenan and carrageenan salts and furcelleran and furcelleran salts from the list of food additives that can safely be added to food are based on the scientific literature in which substantive evidence about the harmful effects of carrageenan has been published. This evidence is deemed to be sufficient, in the view of this petitioner, to justify change in the statutory status of carrageenan, carrageenan salts, furcelleran and furcelleran salts.

In recent months, my colleagues and I have published five substantive scientific papers that detail harmful effects of the common food additive carrageenan on human intestinal cells. These experiments utilize exposures to undegraded carrageenan at concentrations that are markedly less than the anticipated daily human exposure to carrageenan from the diet.

Specific reports include:

1. *Biochimica Biophysica Acta.* 2008 Apr 11. [Epub ahead of print]
Carrageenan-induced NFkappaB activation depends on distinct pathways mediated by reactive oxygen species and Hsp27 or by Bcl10.
Bhattacharyya S, Dudeja PK, Tobacman JK. Department of Medicine, University of Illinois at Chicago, USA.

Carrageenans are highly sulfated polysaccharides that are widely used as food additives due to their ability to improve food texture. They are also widely recognized for their ability to induce inflammation in animal models of colitis. Recently, we reported that carrageenan (CGN) activated a pathway of innate immunity in human colonic epithelial cells mediated by Bcl10 (B-cell CLL/lymphoma 10). However, increases in phospho-IkappaBalpha and Interleukin-8 (IL-8) were not completely inhibited by silencing Bcl10, suggesting that CGN also influenced another mechanism, or mechanisms, of inflammation. In this report, we demonstrate that CGN increases production of reactive oxygen species (ROS) in human colonic epithelial cells. The combination of ROS quenching by the free radical scavenger Tempol and of Bcl10 silencing by siRNA completely inhibited the CGN-induced increases in nuclear NFkappaB (p65), phospho-IkappaBalpha, and secretion of IL-8. The CGN-induced increase in ROS was associated with declines in phosphorylation of MAPK 12 (p38gamma), MAPK 13 (p38delta), and heat-shock protein (Hsp) 27. The CGN-induced decline in phospho-Hsp27 was reversed by co-administration of Tempol (100 nM), but unaffected by silencing Bcl10. Since Hsp27 phosphorylation is inversely associated with phosphorylation of the IkappaBalpha kinase (IKK) signalosome, CGN exposure appears to affect the IKK signalosome by both the catalytic component, mediated by ROS-phospho-Hsp27, and the regulatory component, mediated by Bcl10 interaction with IKKgamma (Nemo). Hence, the CGN-activated inflammatory cascades related to innate immunity and to generation of ROS may be integrated at the level of the IKK signalosome.

2. *Journal of Nutrition.* 2008 Mar;138(3):469-75.
Carrageenan induces cell cycle arrest in human intestinal epithelial cells in vitro.
Bhattacharyya S, Borthakur A, Dudeja PK, Tobacman JK. Department of Medicine, University of Illinois at Chicago and Jesse Brown Veterans Affairs Medical Center, Chicago, IL 60612, USA.

Multiple studies in animal models have shown that the commonly used food additive carrageenan (CGN) induces inflammation and intestinal neoplasia. We performed the first

studies to determine the effects of CGN exposure on human intestinal epithelial cells (IEC) in tissue culture and tested the effect of very low concentrations (1-10 mg/L) of undegraded, high-molecular weight CGN. These concentrations of CGN are less than the anticipated exposure of the human colon to CGN from the average Western diet. In the human colonic epithelial cell line NCM460 and in primary human colonic epithelial cells that were exposed to CGN for 1-8 d, we found increased cell death, reduced cell proliferation, and cell cycle arrest compared with unexposed control cells. After 6-8 d of CGN exposure, the percentage of cells re-entering G0-G1 significantly decreased and the percentages of cells in S and G2-M phases significantly increased. Increases in activated p53, p21, and p15 followed CGN exposure, consistent with CGN-induced cell cycle arrest. Additional data, including DNA ladder, poly ADP ribose polymerase Western blot, nuclear DNA staining, and activities of caspases 3 and 7, indicated no evidence of increased apoptosis following CGN exposure and were consistent with CGN-induced necrotic cell death. These data document for the first time, to our knowledge, marked adverse effects of low concentrations of CGN on survival of normal human IEC and suggest that CGN exposure may have a role in development of human intestinal pathology.

3. *Journal of Biological Chemistry*. 2008 Apr 18;283(16):10550-8. Epub 2008 Feb 5.
Toll-like receptor 4 mediates induction of the Bcl10-NFkappaB-interleukin-8 inflammatory pathway by carrageenan in human intestinal epithelial cells.
Bhattacharyya S, Gill R, Chen ML, Zhang F, Linhardt RJ, Dudeja PK, Tobacman JK. Department of Medicine, University of Illinois, Chicago, Illinois 60612, USA.

The sulfated polysaccharide carrageenan (CGN) induces activation of NFkappaB and interleukin 8 (IL-8) in human colonic epithelial cells through a pathway of innate immunity mediated by Bcl10 (B-cell CLL/lymphoma 10). In this report, we identify Toll-like receptor 4 (TLR4), a member of the family of innate immune receptors, as the surface membrane receptor for CGN in human colonic epithelial cells. Experiments with fluorescence-tagged CGN demonstrated a marked reduction in binding of CGN to human intestinal epithelial cells and to RAW 264.7 mouse macrophages, following exposure to TLR4 blocking antibody (HTA-125). Binding of CGN to 10ScNCr/23 mouse macrophages, which are deficient in the genetic locus for TLR4, was absent. Additional experiments with TLR4 blocking antibody and TLR4 small interfering RNAs showed 80% reductions in CGN-induced increases in Bcl10 and IL-8. Transfection with dominant-negative MyD88 plasmid demonstrated MyD88 dependence of the CGN-TLR4-triggered increases in Bcl10 and IL-8. Therefore, these results indicate that CGN-induced inflammation in human colonocytes proceeds through a pathway of innate immunity, perhaps related to the unusual alpha-1,3-galactosidic linkage characteristic of CGN, and suggest how dietary CGN intake may contribute to human intestinal inflammation. Because CGN is a commonly used food additive in the Western diet, clarification of its effects and mechanisms of action are vital to issues of food safety.

4. *Digestive Diseases and Sciences*. 2007 Oct;52(10):2766-74. Epub 2007 Apr 12.
Carrageenan reduces bone morphogenetic protein-4 (BMP4) and activates the Wnt/beta-catenin pathway in normal human colonocytes.
Bhattacharyya S, Borthakur A, Dudeja PK, Tobacman JK. Department of Medicine, University of Illinois at Chicago and Jesse Brown VA Medical Center, Chicago, Illinois 60612, USA.

Carrageenans are highly sulfated polysaccharides that are widely used as food additives in the Western diet, in order to improve the texture of processed foods. Although native and degraded carrageenans induce colonic ulcerations, polyps, and colorectal tumors in animal models, very little is known about the effects of carrageenan on human colonocytes. We

evaluated effects of lambda-carrageenan (lambdaCGN) on the normal human colonocyte cell line NCM460, using a concentration of 1 ug/ml, significantly less than one tenth the average daily exposure to carrageenan in the Western diet. We measured secreted bone morphogenetic protein-4 (BMP4) in spent media and quantified its expression by quantitative RT-PCR. Wnt-related genes were measured by an oligonucleotide array. Cellular beta-catenin was quantified by ELISA. We found a marked decline in secreted BMP4 ($P < 0.001$) following exposure of NCM460 cells to lambdaCGN for 24 hr. Quantitative RT-PCR for BMP4 transcripts revealed 24% and 45% inhibition of expression on days 2 and 4. cDNA gene expression array of Wnt signaling pathway target genes demonstrated significant changes, including 4.5-fold induction of Wnt 9A and suppression of Dickkopf 3 and RHOU genes. Measurement of beta-catenin by ELISA revealed concomitant accumulation with increases of 67.8%, 61.6%, and 73.9% on days 1, 2, and 4, compared to untreated controls. We conclude that treatment of normal human colonocytes with lambdaCGN activated the Wnt/beta-Catenin cascade and suppressed the expression and secretion of BMP4, inducing significant changes in cellular pathways that are associated with both sporadic and juvenile polyps. CGN may influence development of human intestinal polyps in vivo by these mechanisms.

5. *American Journal of Physiology: Gastrointestinal and Liver Physiology.* 2007 Mar;292(3):G829-38. Epub 2006 Nov 9.
Carrageenan induces interleukin-8 production through distinct Bcl10 pathway in normal human colonic epithelial cells.
Borthakur A, Bhattacharyya S, Dudeja PK, Tobacman JK. Department of Medicine, University of Illinois at Chicago and Jesse Brown Veterans Affairs Medical Center, Chicago, Illinois 60612, USA.

Carrageenan is a high molecular weight sulfated polygalactan used to improve the texture of commercial food products. Its use increased markedly during the last half century, although carrageenan is known to induce inflammation in rheumatological models and in intestinal models of colitis. We performed studies to determine its direct effects on human intestinal cells, including normal human intestinal epithelial cells from colonic surgeries, the normal intestinal epithelial cell line NCM460, and normal rat ileal epithelial cells. Cells were treated with high molecular weight lambda-carrageenan at a concentration of 1 ug/ml for 1-96 h. IL-8, IL-8 promoter activity, total and nuclear NF-kappaB, IkappaBalpha, phospho-IkappaBalpha, and Bcl10 were assessed by immunohistochemistry, Western blot, ELISA, and cDNA microarray. Increased Bcl10, nuclear and cytoplasmic NF-kappaB, IL-8 promoter activation, and IL-8 secretion were detected following carrageenan exposure. Knockdown of Bcl10 by siRNA markedly reduced the increase in IL-8 that followed carrageenan exposure in the NCM460 cells. These results show, for the first time, that exposure of human intestinal epithelial cells to carrageenan triggers a distinct inflammatory pathway via activation of Bcl10 with NF-kappaB activation and upregulation of IL-8 secretion. Since Bcl10 contains a caspase-recruitment domain, similar to that found in NOD2/CARD15 and associated with genetic predisposition to Crohn's disease, the study findings may represent a link between genetic and environmental etiologies of inflammatory bowel disease. Because of the high use of carrageenan as a food additive in the diet, the findings may have clinical significance.

These reports expand on previous conclusions in the literature that reported inflammation, neoplasms, and ulcerations caused by carrageenan exposure in animal models. Other reports have identified carrageenan as a cause of allergy and anaphylaxis, and carrageenan has been extensively used to induce inflammation in experimental models.

Recent findings that lead to the current petition are summarized by the following:

1. Exposure of human colonic epithelial cells in tissue culture to small quantities of undegraded carrageenan produced inflammatory responses, including activation of the important inflammatory mediator NFκB and increased secretion of the important chemokine IL-8, which signals for development of an inflammatory infiltrate.
2. Exposure of human colonic epithelial cells in tissue culture to small quantities of undegraded carrageenan produced an increase in cell death with cell cycle arrest, effects that can contribute to ulcerations.
3. Exposure of human colonic epithelial cells in tissue culture to small quantities of undegraded carrageenan was associated with changes in wnt and BMP4 that resemble the changes found in human colonic polyps.
4. Exposure of human colonic epithelial cells in tissue culture to small quantities of undegraded carrageenan was associated with a pathway of innate immunity, consistent with the unusual chemical structure of carrageenan, including the alpha-1,3-galactosidic linkage that is a known antigenic epitope in humans.
5. Exposure of human colonic epithelial cells in tissue culture to small quantities of undegraded carrageenan produced inflammation by a second pathway of reactive oxygen species, as well as by the innate immune pathway.

The carrageenan used in the majority of the experiments was high molecular weight (at least >100,000), undegraded, lambda-carrageenan obtained from Sigma-Aldrich. The concentration of carrageenan used in the majority of our experiments was 1 microgram / ml. In the human intestine, if 100 mg / day of carrageenan is consumed, in an intestinal contents of 3.5 liters, this would be a concentration of 100 mg / 3500 ml or almost 29 micrograms / ml, significantly greater than the concentration used in our experiments. 100 mg / day is probably a low estimate of current carrageenan intake, and is based on studies performed in the United States in the 1970s when there were fewer processed foods that included carrageenan.

Similarities between the chemical structure of carrageenan, carrageenan salts, furcelleran, and furcelleran salts justify the extension of this petition to include each of these categories.

Harmful effects of carrageenan have often been attributed to the low molecular weight forms of carrageenan or to possible contamination by lipopolysaccharide. As stated, our studies report findings with high molecular weight carrageenan. We excluded contamination of carrageenan by lipopolysaccharide (LPS) by testing responses to carrageenan in the presence of polymyxin B which inhibits LPS. In vivo, it is highly likely that high molecular weight carrageenan will break down to lower molecular weight forms by stomach acid, mechanical processes of digestion, effects of colonic bacteria, or heat. Also, food manufacturers routinely find contamination of undegraded carrageenan used in food processing by lower molecular weight forms. However, as stated, harmful effects of undegraded carrageenan were demonstrated by our experiments with human colonic epithelial cell lines, ex vivo human colonic tissue, ex vivo mouse colonic tissue, and in the in vivo mouse model.

We regard these findings as compelling evidence of the inflammatory effects and other harmful effects associated with carrageenan exposure and petition the FDA to remove carrageenan and carrageenan salts, and the related furcelleran and furcelleran salts from the list of food additives that can be safely used in food.

C. Enviromental Impact Statement

There is categorical exclusion from an Environmental Impact Statement based on petition for revocation of administrative regulation [25.30 (h)].

D. Economic Impact Statement

Not required at this time.

E. Certification

The undersigned certifies that, to the best knowledge and belief of the undersigned, this petition includes all information and views on which the petition relies, and that it includes representative data and information known to the petitioner which are unfavorable to the petition.

Joanne K. Tobacman, M.D.
(Signature)

Joanne K. Tobacman, M.D
Name of Petitioner

(Mailing Address)