DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
College Park, MD 20740

JUN 11 2012

Joanne K. Tobacman, M.D.
(b) (6)

Re: Docket No. FDA-2008-P-0347

Dear Dr. Tobacman:

This responds to your citizen petition received on June 11, 2008, requesting that the Food and Drug Administration (FDA) revoke the regulations in Title 21, Code of Federal Regulations (CFR) 172.620, 172.655, 172.626, and 172.660 that permit the use of carrageenan, furcelleran, and their salts for direct addition to food.[1]

Your petition asserts that such action is warranted based on results from five studies (Refs. 1-5) that you claim show that carrageenan induces intestinal inflammation and intestinal neoplasia. In addition your petition further asserts that furcelleran is structurally similar to carrageenan, the results of the carrageenan studies warrant your requested action regarding furcelleran and its salts.[2] Your petition also asserts that other literature has reported inflammation, neoplasms, and ulcerations caused by carrageenan exposure in animal models. Finally, you raise other general safety concerns regarding carrageenan.

In accordance with § 10.30(e)(3) this letter is to advise you that FDA is denying your petition for the reasons stated below.

1. Experimental model systems used in the studies are not representative of effects from approved uses

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[1] Pursuant to section 409(b)(1) of the Federal Food, Drug, and Cosmetic Act (the Act) [21 U.S.C. § 348(b)(1)], FDA, acting on a petition, may, by order, establish a regulation prescribing, with respect to one of more proposed uses of a food additive, the conditions under which such additives may be safely used. FDA regulations pertaining to establish a food additive regulation based on a petition may be found at 21 CFR Part 171.

FDA prescribes the conditions under which carrageenan, furcelleran and their salts may be used in foods in 21 CFR 172.620, 172.655, 172.626, and 172.660. FDA promulgated the regulations based on FDA's review of petitions submitted by Marine Colloids, Inc., and T. M. Duche and Sons, Inc. and other relevant material, 26 FR 9411, 9411-12 (October 6, 1961). The regulations established that the food additive substances, as defined in the respective regulations, may be 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.

[2] We note that FDA has recognized that the similarities between carrageenan and furcelleran and their salts, and in 1996 requested comment on a proposal to consolidate the respective food additive regulations into one regulation for carrageenan. 61 FR 29701 (June 12, 1996).

In your petition, you request that FDA revoke the regulations in 21 CFR 172.620, 172.655, 172.626, and 172.660 based on the results of the five publications discussed in your petition. You summarize the findings from the five referenced studies that lead to your petition as follows:

- 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.
- 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.
- 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.
- 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.
- 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.

We have reviewed the publications you submitted with your petition to determine if they provide evidence that supports your request. We note that all studies discussed in the five submitted research papers on carrageenan used *in vitro* cell/tissue culture models such that the colonic cells were directly exposed to carrageenan, and conclusions were based on cellular changes measured by biochemical or molecular biological techniques. Such models do not provide a parallel to food consumption (e.g., intake without food components, such as proteins). Such a model offers limited value to the safety evaluation of a substance as a dietary component.

For example, in the studies referenced in your petition, the contact time between the individual intestinal cells and carrageenan in the static cell culture media under the test conditions was the incubation time in the experiment (generally 24 and 48 hours). FDA concludes that such exposure conditions are likely to represent tissue edema-induced inflammation models rather than exposure to carrageenan from use as a food ingredient.

In particular, some of your conclusions based on the submitted publications support the findings in previous studies with an *in vivo* edema/inflammation model. For instance, generation of reactive oxygen species (ROS) and subsequent activation of NF-κB signaling, and dependence on Toll-like receptor 4 and/or MyD88 in cellular response to carrageenan (Refs. 1 & 3) were well-documented phenomena in a carrageenan-induced acute tissue edema/inflammation model (Refs. 6 & 7).

Thus, FDA concludes that the data in the studies referenced in your petition are relevant to routes of exposure for carrageenan such as subcutaneous injection in a paw edema model, but not to exposure from human consumption of food containing the direct food additives in question. We note that the regulations prescribing the allowable uses of carrageenan, furcelleran and their salts permit their use on or in food for human consumption. FDA considers the administration of undegraded carrageenan as a part of the diet of animals to be a more appropriate experimental model for evaluating the safety of exposure to carrageenan through consumption in humans. Therefore, FDA's safety evaluation of the allowable uses of carrageenan, furcelleran, and their salts was based on studies using oral administration models. FDA's evaluation of such studies has confirmed the safety of carrageenan for use as a food ingredient. Your petition does not provide support for using *in vitro* cell/tissue culture models to evaluate carrageenan, furcelleran, and their salts.[3]

2. References to reports in other literature of inflammation, ulcerations, and neoplasms

Your petition states that the studies submitted "expand on previous conclusions in the literature that reported inflammation, neoplasms, and ulcerations caused by carrageenan exposure in animal models."

Regarding inflammation and ulcerations, your petition does not provide references to these studies, and FDA finds that the existing literature does not provide support for your requested actions. FDA has previously considered the inflammatory and ulcerogenic effects of carrageenan in animals, and has found that studies demonstrating such effects do not support revocation of regulations permitting its use. For example, FDA addressed animal models demonstrating ulcerative effects on guinea pigs and rabbits in a response dated March 26, 1981 to a previous citizen petition on carrageenan, (Docket No. FDA-1980-P-0336/CP). In that response, FDA stated that studies involving degraded or low molecular weight carrageenan in susceptible species using non representative dosage forms did not support revocation of 21 CFR 172.620. Our current evaluation of the safety of carrageen as a food additive in response to your petition affirms FDA's previous conclusion that such animal models are not representative of the inflammatory and ulcerative effects in humans of the approved uses of carrageenan, and thus do not support the revocation of 21 CFR 172.620. (Refs. 8 & 9).

Regarding neoplasms and tumor promotion, your 2001 review paper cited several early studies that implicated tumor-promoting activity of undegraded carrageenan (Ref. 10). Those studies support that carrageenan lacks tumor-initiating activity, but increases the occurrences of

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[3] In addition, we note that the results from the *in vitro* mechanistic studies provide conflicting results regarding cell responses to carrageenan. For example, in one study, the treatment of colonic cells with carrageenan resulted in the activation of a signaling pathway considered to promote proliferation in either normal intestinal crypt development or hyperplasia during tumorigenesis (Ref. 7). However, under identical experimental conditions, another study that performed cell cycle progression analyses showed that the treatment of colonic cells with carrageenan resulted in cell cycle arrest rather than promotion of cell cycle progression (Ref. 2). Thus, FDA concludes that the limited value of the studies due to the experimental model employed is further diminished by the lack of consistent conclusions from the study results.

intestinal tumor or aberrant crypt foci initiated by known mutagens, such as azoxymethane or methylnitrosourea, and 1,2-dimethylhydrazine. However, the findings of these studies have been disputed (Ref. 11). In addition, the classical initiation-promotion study in rat colon with 1,2-dimethylhydrazine did not show any effect of carrageenan on tumor formation up to a concentration of 5% (equivalent to 3229 mg/kg/day) (Ref. 12). Furthermore, although one study showed an enhancing effect of carrageenan on azoxymethane-initiated aberrant crypt foci growth in the rat colon (Ref. 13), a published follow-up study showed that the facilitation of aberrant crypt foci growth depends on rat-specific microflora and is not observed in germ-free rats inoculated with adapted microflora from fecal samples taken from healthy children given carrageenan-containing desserts 3 times a week for 3 weeks (Ref. 14). Therefore, FDA concludes that the available research does not demonstrate that carrageenan induces tumors.

3. General safety concerns raised regarding the safety of carrageenan as a food additive

Your petition raised other concerns (italicized below) about using carrageenan as a food additive. Our responses to these issues are as follows:

"Harmful effects of carrageenan have often been attributed to the low molecular weight forms of carrageenan… 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."

The possibility of carrageenan degradation in the stomach was raised by a very early study, based on an *in vitro* experimental system with a simulated gastric juice of pH 1.9 (Ref. 15). However, more carefully designed *in vitro* or *in vivo* studies published later failed to confirm extensive carrageenan breakdown. One study that used a more elaborate artificial stomach simulation system demonstrated that the weight-average molecular weight of the majority of carrageenan remains greater than 100 kDa (Ref. 16). An *in vivo* experiment using gel-filtration chromatography analysis showed that the amounts and the molecular weight distribution of fecal carrageenans are almost identical to those carrageenans fed to rats (Ref. 17). Another recent study also demonstrated that the average molecular weight of carrageenan was not significantly changed during its digestive transit in rats fed undegraded κ-carrageenan (345,000 mol wt) (Ref. 14).

Further, pursuant to 21 CFR 172.5(a), regulations prescribing the conditions under which food additive substances may be safely used require usage under good manufacturing practice.[4] The results of a recent survey that investigated molecular weight distributions of 29 food-grade carrageenan samples found that average molecular weight of carrageenans ranged from 453 to 652 kDa with a mean of 530 kDa (Ref. 18). Importantly, this analysis did not detect low

[4] FDA has previously supported the selection of 100,000 as an average molecular weight minimum for food-grade carrageenan. *See* 44 FR 40343, 40344 (July 10, 1979).

carrageenan samples found that average molecular weight of carrageenans ranged from 453 to 652 kDa with a mean of 530 kDa (Ref. 18). Importantly, this analysis did not detect low molecular weight, degraded product (i.e., poligeenan with a molecular weight of 20-30 kDa) in the carrageenan samples with a detection limit for poligeenan of approximately five percent (weight/weight).

"Other reports have identified carrageenan as a cause of allergy and anaphylaxis…"

You did not provide specific references to support this statement. Our search of the published literature did not reveal any publication that unequivocally supports this statement. Through a literature search, FDA found two relevant publications on the effects of carrageenan ingestion on allergic reactions and oral tolerance with mouse food allergy models. Frossard et al. (2001) showed that ingestion of carrageenan significantly reduced the incidence of anaphylaxis and other allergic responses in C3H/HeJ mice sensitized to a cow's milk protein (β-lactoglobulin) (Ref. 19). Similar results were obtained by Tsuji et al. (2003) (Ref. 6). In this study, oral administration of low-dose carrageenan (0.001 – 0.005%) significantly decreased the levels of serum histamine and antigen (ovalbumin)-specific serum IgE. Importantly, carrageenan ingestion did not decrease passive cutaneous anaphylaxis or production of antigen-specific serum IgG_1 and IgG_{2a} in these two studies, indicating that carrageenan consumption would not cause nonspecific immune suppression. It should be noted that the core finding of one of the submitted papers (Ref. 3), the activation of TLR4/MyD88 signaling pathway in colonic cells directly exposed to carrageenan in the cell culture dish, was also observed with the *in vitro* primary culture of immune cells as well as the *in vivo* foot edema model (Ref. 20). However, it appears that suppression of allergic responses and promotion of oral tolerance by oral ingestion of carrageenan are TLR4-independent (Ref. 6). We note further that under FDA's regulations prescribing their allowable uses, carrageenan, furcelleran, and their salts must be declared when used as ingredients in food.

Conclusion

For the reasons stated above, FDA concludes that your petition does not adequately support your requested action. Therefore, FDA is denying your petition to revoke the regulations that permit the use of carrageenan, furcelleran, and their salts for direct addition to food.

Sincerely,

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Ted Elkin
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