EVALUATION OF THE HEALTH ASPECTS OF CARRAGEENAN

AS A FOOD INGREDIENT

June 1973

Prepared for

Bureau of Foods
Food and Drug Administration
Department of Health, Education, and Welfare
Washington, D. C.

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Life Sciences Research Office
Federation of American Societies for Experimental Biology
9650 Rockville Pike
Bethesda, Maryland 20014
NOTICE

This report is one of a series of evaluations of the health aspects of the Generally Recognized as Safe (GRAS) food substances that are being made by the Life Sciences Research Office (LSRO) of the Federation of American Societies for Experimental Biology (FASEB) under contract with the Food and Drug Administration (FDA) of the U. S. Department of Health, Education, and Welfare. The Federation recognizes that the safety of GRAS substances is of national significance, and its resources are particularly suited to marshalling the opinions of knowledgeable scientists to assist in these evaluations. The Life Sciences Research Office, established in 1962 to make scientific assessments in the biomedical sciences, is conducting these studies.

Qualified scientists were selected as consultants to make a continuing review, analysis, and evaluation of the available information on each of the GRAS substances. These scientists, designated the Select Committee on GRAS Substances, were chosen for their competence and judgment with due consideration for balance and breadth in the appropriate professional disciplines. Members of the Select Committee on GRAS Substances who have contributed to this report are named in Section VII. The Select Committee's evaluations are being made independently of FDA or any other governmental or nongovernmental group.

These reports are approved by the Select Committee prior to submission to FDA. Although most LSRO consultants are members of FASEB constituent societies, the reports do not necessarily reflect the views of the Federation as a corporate body or carry the endorsement of the members of its constituent societies.

C. Jelleff Carr, Ph.D., Director
Life Sciences Research Office
FASEB
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I. INTRODUCTION

Under terms of FDA Contract 72-85, FASEB's Life Sciences Research Office was requested to evaluate the health aspects of using carrageenan as a food ingredient, primarily on the basis of information contained in a monograph furnished by FDA (1), summarizing the world's scientific literature from 1920 through 1970, and in certain supplemental documents available as of June 1973. Carrageenan is one of the food substances that has been generally recognized as safe (GRAS) under the provisions of Section 121.101 of the Code of Federal Regulations (21 CFR 121.101, revised January 1, 1972).

As indicated in the Food, Drug and Cosmetic Act [21 USC 321(s)], GRAS substances are exempt from the requirement of prem­arketing clearance for food additives. It is stated in 21 CFR 121.1 that GRAS means general recognition of safety by experts qualified by scientific training and experience to evaluate the safety of substances on the basis of scientific data derived from published literature. This section of the Code also indicates that expert judgment is to be based on the evaluation of results of credible toxicological testing, or for those substances used in food prior to January 1, 1958, on a reasoned judgment founded in experience with common food use, and is to take into account reasonably anticipated patterns of consumption, cumulative effects in the diet, and safety factors appropriate for the utilization of animal experimentation data. It is recognized further (21 CFR 121.3) that it is impossible to provide assurance that any substance is absolutely safe for human consumption.

The Select Committee on GRAS Substances of LSRO is making its evaluations of these substances in full recognition of the foregoing provisions. In reaching its conclusions on safety, the Select Committee, in accord with FDA's guidelines, is relying primarily on the absence of substantive evidence of a significant risk to the public health, and realizes that a decision, based on reasoned judgment, is expected even in instances where the available information is qualitatively, or quantitatively limited. The Committee is also aware that biological testing, like all of science, is dynamic. Accordingly, the Committee's decisions, based as they are on the information now available, cannot anticipate and be guided by experiments not yet done or by the results of tests that may be reconducted, using new technologies that are constantly being evolved. These decisions will need to be reviewed as new or better information becomes available.
In this context, the LSRO Select Committee on GRAS Substances has reviewed the available information on carrageenan and submits its interpretation and assessment in this report, which is intended for the use of FDA in determining the future status of carrageenan under the Federal Food, Drug, and Cosmetic Act.

II. BACKGROUND INFORMATION

Carrageenan, also known as Irish moss, is a mixture of calcium, sodium, potassium, ammonium, and perhaps other salts of a sulfated polysaccharide consisting predominantly of galactose and anhydrogalactose units. It is prepared from aqueous extracts of several red marine algae of the families Gigartinaceae and Soleraceae, class Rhodophyceae, including Chondrus crispus (the most important source in the U. S.) and Gigartina sp. and Euchema sp. which are also important sources of European carrageenan. Major sources for the U. S. are maritime Maine, Massachusetts, and Canada; for Europe they are Ireland, France, and Norway. Raw materials from Spain, Portugal, and North Africa are also used by both American and European processors. The weed is harvested by hand raking from small boats at ebb tide, dried, baled and shipped to processors. It is washed in cold water, can be ion-exchanged if monovalent salts are desired in the product, and the carrageenan is extracted with hot water, filtered, treated with adsorbents to remove soluble impurities, and either drum dried or precipitated with alcohol and then dried (1, 2).

Commercial carrageenan varies in structure and composition depending on the source and subsequent processing procedures. Two broadly characterizable types of carrageenan exist. The first, now generally designated "undegraded carrageenan," is derived chiefly from Chondrus sp., is of average molecular weight 100,000 or more, and is regarded as consisting of two major fractions which may be separated by precipitation with potassium ions; the precipitated fraction, designated \( \kappa \)-carrageenan (about 60 percent), possesses gelling properties while the soluble fraction designated \( \lambda \)-carrageenan (about 40 percent), is non-gelling. The second broad type is of average molecular weight 10,000 or less, is non-gelling, and consists largely of \( \iota \)-carrageenan (2). Products that are naturally of low molecular weight or that have been degraded by hydrolysis are now generally designated "degraded carrageenan."
The Food Chemicals Codex (3) specifies tests for gelling behavior of food grade carrageenan and limits of not more than 35 percent ash, 1.0 percent acid insoluble ash, 12 percent moisture, 3 ppm arsenic, 40 ppm heavy metals as lead, 10 ppm lead, and 20 to 40 percent sulfate on a dry weight basis.

The pertinent entry in the GRAS list in the Code of Federal Regulations (4) is "Chondrus extract (carrageenin)." By amendment in 1972 (5) the GRAS list item "Chondrus extract (carrageenin)" was proposed to be deleted, and the following specification added to Section 121.1066(b) to assure that carrageenan as used in foods in the U. S. is of high molecular weight: "It (carrageenan) has a minimum viscosity in 1.5 percent-by-weight aqueous solution of 5 centipoises at 75°C., as determined by LVF-series Brookfield viscometer using a UL (ultra low) adapter at 30 r.p.m. (or by other equivalent method), representing an average molecular weight exceeding 100,000." Section 121.1066 defines the conditions for safe use of the food additive carrageenan; Section 121.1067 provides similar information for salts of carrageenan (6).

The only product considered in this report is the undegraded, high molecular weight carrageenan which is, as noted above, the only type of carrageenan on the GRAS list.

The use of dried Irish moss in food and medicinals has been practiced for centuries in Ireland. It began to be used elsewhere, including the U. S., in the early 19th century, but commercial carrageenan was not available until the World War II period when it was produced as a substitute for agar. Carrageenan is used by the food industry as an emulsion stabilizer because of its viscosity, gelling properties and unique capacity to react with proteins (2). Particular advantage is taken of the latter property in preparing stable suspensions of cocoa or other particles in milk, for example, where carrageenan forms complexes with casein that are not precipitated by calcium ions (7). In practical food applications the gelling properties of carrageenan are sometimes modified by adding other hydrocolloids such as carob bean gum (2).

Carrageenan is present in amounts ranging from 1.13 to 0.005 percent in the following categories of foods arranged in decreasing order of carrageenan content: Jams and jellies, sweet sauces (0.4 percent to 0.2 percent), toppings and frostings, gelatins and puddings, poultry (0.12 percent), condiments and relishes, alcoholic beverages, milk products (0.1 to 0.06 percent), imitation dairy products, baked
goods, soft candy, processed fruits, nonalcoholic beverages (0.04-
0.03 percent), baby formulas (0.03 percent), soups, frozen dairy
products, and cheese (8).

The total poundage of carrageenan used in foods approximately
doubled between 1960 and 1970 (8). However, there is no information
available to the Select Committee that permits it to determine the
extent to which there has been any significant change in the carrageenan
content of the foregoing food categories over the past decade.

III. CONSUMER EXPOSURE DATA

A National Research Council subcommittee has provided the
following information on the possible daily human intake of carrageenan
in the total diet by individuals in various age groups (8). The Select
Committee has converted these figures to possible intakes per kilogram
of body weight.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Possible daily intake</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Per kilogram of body weight*</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>Maximum</td>
</tr>
<tr>
<td>0-5 mos.</td>
<td>100</td>
<td>184</td>
</tr>
<tr>
<td>6-11 mos.</td>
<td>197</td>
<td>688</td>
</tr>
<tr>
<td>12-23 mos.</td>
<td>218</td>
<td>544</td>
</tr>
<tr>
<td>2-65+ yrs.</td>
<td>367</td>
<td>917</td>
</tr>
</tbody>
</table>

*Calculations based on an average weight of 60 kg for an adult (9)
and the following estimated weights of infants by age groups: 0-5 mos.,
5 kg; 6-11 mos., 8 kg; and 12-23 mos., 11 kg (10).

It is recognized that in the age group 2-65+ years, the figures
for daily intake of carrageenan per kilogram of body weight could be
low for some, since most individuals from age 2 to maturity will weigh
less than 60 kg; thus the daily intake of carrageenan by a 20 kg child
could be higher by a factor of 3 than the figures indicated in the table.
It is to be noted that the NRC subcommittee states (8) that its calculated intakes of GRAS substances are overstated in most cases, often by considerable margins.* This is likely to be true in the case of carrageenan as the following calculations indicate.

The average daily per capita intake of 367 mg of carrageenan given in the foregoing table would require the use in food of some 27 million kg of carrageenan per year in the U.S. Other NRC data (8) indicate that only 2.1 million kg were reported by the food industry to be added to food in 1970. Use of this smaller amount would permit a daily average per capita intake of only 29 mg. An independent, and even lower, estimate (11) indicates that U.S. sales of carrageenan for food uses have varied between 1.42 million kg and 1.68 million kg per year over the past 5 years. Based on the highest of these figures, the per capita daily average intake would not exceed 23 mg.

In the light of these considerations, therefore, the Select Committee regards the figures given in the table as consumption levels that are highly unlikely to be achieved by any of the age groups.

The FAO/WHO Expert Committee on Food Additives (12) estimates an acceptable daily intake of carrageenan as 0 to 500 mg per kg body weight.

IV. BIOLOGICAL STUDIES

There are no significant studies on the long-term feeding of undegraded, high molecular weight carrageenan (more than half the life span of the species). The following short-term studies are relevant.

The oral LD₅₀ of calcium carrageenan in g/kg is: mouse, 9.2; hamster, 6.8; rat, 5.4; and rabbit, 2.6. The corresponding values for

*An explanation for such overstatements is detailed in Section XI, "Significance and Use of Data in Safety Evaluations," of the NRC subcommittee's report (8). The Select Committee finds this explanation reasonable and concurs in the first recommendation in Section XII of the same report, that "In order to conduct a more accurate survey of the intake of substances used in food processing, food consumption data collected specifically for this purpose are needed."
sodium carrageenan are: 9.2, 8.0, 6.0, and 5.0 (13, 14).

Studies in a number of species, including man, indicate that very little, if any, carrageenan is absorbed from the gastrointestinal tract; most is eliminated in the feces (15, 16-19). Gerbils, rats, and guinea pigs were clinically unaffected when fed carrageenan for up to six months in the diet or in drinking water in amounts ranging from 1 to 4.2 g per kg per day (18). No effect was noted on the integrity of the gastrointestinal epithelium. Rhesus monkeys given 1 percent carrageenan in drinking water (corresponding to 1.3 g per kg per day) for 7 to 11 weeks, were clinically unaffected as compared to the control animals (18). No gross or histopathological change was visible in the gastrointestinal tract at autopsy, even in animals to which carrageenan was subsequently administered by stomach tube in doses increasing to 1.25 g per kg per day for a total of 84 days. When Danish Landrace pigs were fed undegraded carrageenan of molecular weight about 200,000 for 83 days at levels of 50, 200, and 500 mg per kg per day no adverse effects were observed in behavior, growth, food utilization, hematology or blood and urine parameters. No ulcerative changes and erosions of the caecal and colonic mucosae were detected (20).

On the other hand, adverse effects have been reported. Rats fed carrageenan at levels of 5 to 20 g per kg per day in the diet in lieu of starch showed slowing of growth rate (16). Guinea pigs receiving 1 percent carrageenan (identified as "undegraded carrageenan derived from E. spinosum") in drinking water corresponding to a level of about 1.5 g per kg per day for 20 to 30 days, exhibited ulcerative colitis (21). It is to be noted that while the report does not record the molecular weight of the carrageenan used, Eucheuma sp. usually yield carrageenan having a molecular weight of 10,000 or less.

Recent investigations generally confirm the susceptibility of the guinea pig to ulcerative colitis when fed carrageenan at a level of 5 percent in the diet. This susceptibility does not appear to exist in the rat, mouse, hamster, pig, squirrel monkey, and man (20, 22). These studies strongly suggest that the occurrence of ulcers in the large bowel of animals is a species-specific phenomenon.

Fetotoxic effects have been reported for both sodium (23) and calcium carrageenan (24). The oral administration of calcium carrageenan in corn oil, in graded doses up to 900 mg per kg to pregnant mice beginning on day 6, and continuing through day 15 of gestation caused an apparent increase in the number of resorptions and/or fetal deaths in utero. There was a corresponding decrease in the number
of live young and a reduction in weight at delivery, both of which appear to have been dose-dependent. A concurrent retardation in skeletal maturation was inferred from the increased incidence of missing sternebrae and incomplete skull closure. It was concluded that calcium carrageenan is fetotoxic in the pregnant mouse without exhibiting frank teratogenicity. Under the same experimental conditions, rats administered up to 600 mg per kg of calcium carrageenan beginning on day 6 through day 15 of gestation, showed an apparent increase in the number of resorption sites with or without a corresponding decrease in the number of live young. A concurrent retardation in skeletal maturation was indicated by a dose-dependent increase in missing sternebrae. It was concluded that calcium carrageenan depressed fetal development in the pregnant rat and caused an increase in early fetal deaths without evidence of frank teratogenicity. On the other hand, oral administration of graded doses of calcium carrageenan up to 600 mg per kg from day 6 through day 10 of gestation in hamsters, and of graded doses up to 260 mg per kg from day 6 through day 18 in rabbits, had no clearly significant effect on nidation or on maternal or fetal survival. There was some evidence of delayed skeletal maturation which appeared to be dose-dependent. Nearly identical results were obtained in the same four animal species with sodium carrageenan (23).

Some results of ongoing teratology tests on calcium and sodium carrageenan are available (25). Pregnant Osborne-Mendel rats intubed with 10 to 200 mg per kg per day of calcium carrageenan in water on day 6 through day 15 of gestation, exhibited no terata but 4 of 220 young showed gastrointestinal hemorrhages at the 100 mg per kg level. Such hemorrhages were not discernible at higher and lower dosages. There was no significant effect on the number of live fetuses per litter or on weight of live fetuses. Resorptions per litter tended to be slightly higher with carrageenan than without it. When calcium carrageenan was administered in corn oil under the same experimental conditions there was no significant effect on the number of live fetuses per litter, on weight of fetuses, or on resorptions per litter. Terata were observed in 1.23 percent (4 of 324 fetuses) of the fetuses when dams received 300 mg per kg of calcium carrageenan daily. No terata were observed at the 600 mg per kg daily dosage level. Abnormalities included one animal with exencephaly, two animals with fused ribs, and one animal with partial acrania, exencephaly, anophthalmia, atelostomia, beaked nose, fused ribs, and edema. In parallel experiments using sodium carrageenan, no significant differences were observed between experimental animals and controls. Experiments using even higher levels of both sodium and calcium carrageenan in corn oil are not yet available.
A study has been conducted of mutagenic effects of calcium carrageenan (26). In the host-mediated assay, there were no measurable mutagenic responses or alterations in the recombination frequency of Saccharomyces cerevisiae either in vivo or in the associated in vitro tests. In the dominant lethal test there were no consistent responses ascribable to mutagenicity. In the cytogenetic assay, calcium carrageenan produced a slight adverse effect on metaphase chromosomes of rat bone marrow. Four percent of the cells in rats receiving 5 g per kg calcium carrageenan exhibited aberrant chromosomes compared to 1.3 percent in the controls and 10 percent in animals receiving the positive reference compound, triethylenemelamine, at a level of 0.5 mg per kg.

Oral studies of carcinogenicity have not been reported. However, an experiment designed to demonstrate the carcinogenic activity of imines, showed increased incidence (about 20 times that anticipated) of sarcomas in the flanks of rats that were injected only with a 1 percent "carrageenin" solution which was being used as the control (27). The report does not indicate whether the "carrageenin" used was degraded or undegraded.

A number of reports concerning cytotoxic effects of carrageenan are noteworthy in that they could be of significance if circumstances should be such that appreciable absorption of orally administered carrageenan should occur during periods of infectious challenge or metabolic disease. Carrageenan is reported to exert cytotoxic effects on macrophages in vitro (28, 29) and to cause suppression of delayed hypersensitivity reactions in tuberculin sensitive guinea pigs (30). Carrageenan is also reported to activate the Hageman factor causing procoagulant activity in human platelets in vitro (31), increased vascular permeability, and liberation of kinin in vitro (31, 32). Finally, carrageenan is reported to inhibit the activity of complement by interfering with its binding capacity of C1 in vitro and in vivo (33,34, 35). It is impossible to ascertain in any of the foregoing experiments the exact nature of the carrageenan used.

There is no reported evidence to indicate that carrageenan constitutes an allergenic hazard.

V. OPINION

The available information on the oral administration of undegraded carrageenan at levels greatly exceeding the daily human intake, reveals
evidence of possible adverse effects on the gastrointestinal epithelium. Extensive recent investigations of carrageenan and the pathogenesis of gastrointestinal changes indicates the susceptibility of the guinea pig to ulcerative colitis when fed relatively high levels of carrageenan in the diet. This work suggests that the occurrence of ulcers in the large bowel of animals is a species-specific phenomenon where feeding of carrageenan can induce ulceration in the caecum and proximal colon of the guinea pig which to date, does not appear to occur in the rat, mouse, hamster, pig, squirrel monkey, or man.

Recent reports on the oral administration of undegraded sodium and calcium carrageenan of known quality to pregnant animals reveals fetotoxic effects, with or without frank teratogenic effects, in some species at levels that do not greatly exceed the average daily human rate of intake. These effects appear to be dose-dependent.

While carrageenan exhibits no mutagenic effects as measured by the host-mediated and dominant lethal assay procedures, significant abnormalities appear to be induced in the anaphase figures of human embryonic lung cells in tissue culture at dosages that are slightly above average daily human intake.

It is of further concern that parenterally administered carrageenan is reported to inhibit the activity of complement, exert cytotoxic effects on macrophages, suppress delayed hypersensitivity reactions in some tuberculin sensitive animals, activate factors causing procoagulant activity in human blood platelets, increase vascular permeability, and liberate kinin *in vitro*, all of which point to the possibility of the generation of toxic effects that could cause adverse responses following the oral consumption of carrageenan if, during pregnancy or in the presence of infectious challenge or metabolic disorder, appropriate amounts of carrageenan should be absorbed from the gastrointestinal tract.

The Select Committee has been informed that additional animal feeding and teratologic studies are soon to be initiated on commercial carrageenan and on several of the separated polysaccharide components of carrageenan. The Committee's opinion should be reviewed once the results of these studies become available.
The Select Committee has weighed the foregoing and concludes that:

While no evidence in the available information on undegraded carrageenan demonstrates a hazard to the public when it is used at levels that are now current and in the manner now practiced, uncertainties exist requiring that additional studies should be conducted.
VI. REFERENCES CITED


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VII. SCIENTISTS CONTRIBUTING TO THIS REPORT

1. Members of the Select Committee on GRAS Substances:

Aaron M. Altschul, Ph.D., Professor, Department of Community Medicine and International Health, School of Medicine, Georgetown University, Washington, D. C.

Joseph F. Borzelleca, Ph.D., Professor of Pharmacology, Medical College of Virginia, Health Sciences Division, Virginia Commonwealth University, Richmond, Va.

Bert N. La Du, Jr., M.D., Ph.D., Professor and Chairman, Department of Pharmacology, New York University School of Medicine, New York, N. Y.

John R. McCoy, V.M.D., Professor of Comparative Pathology, New Jersey College of Medicine and Dentistry, Rutgers Medical School, New Brunswick, N. J.

Sanford A. Miller, Ph.D., Professor of Nutritional Biochemistry, Massachusetts Institute of Technology, Cambridge, Mass.

Gabriel L. Plaa, Ph.D., Professor and Chairman, Department of Pharmacology, University of Montreal Faculty of Medicine, Montreal, Canada.

Ralph G. H. Siu, Ph.D., Consultant, Washington, D. C.

John L. Wood, Ph.D., Distinguished Service Professor, Department of Biochemistry, University of Tennessee Medical Units, Memphis, Tenn.

George W. Irving, Jr., Ph.D. (Chairman), Research Associate, Life Sciences Research Office, Federation of American Societies for Experimental Biology, Bethesda, Md.
2. LSRO staff:

C. Jelleff Carr, Ph.D., Director, LSRO/FASEB.
Samuel B. Detwiler, Jr., Research Associate, LSRO/FASEB.
Kenneth D. Fisher, Research Associate, LSRO/FASEB.
Andrew F. Freeman, Research Associate, LSRO/FASEB.

3. Ad hoc consultant:

Henry Stevens, Ph.D. (USDA Retired), Consultant on Allergens,
Washington, D.C.

Report submitted by:

June 12, 1973
Date

George W. Irving, Jr., Chairman
Select Committee on GRAS Substances