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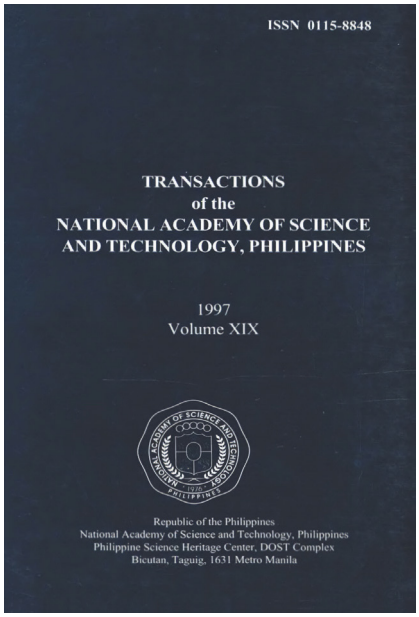

Toxicological Studies on Philippine Natural Grade Carrageenan

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TOXICOLOGICAL STUDIES ON PHILIPPINE NATURAL GRADE CARRAGEENAN

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ABSTRACT

PNG carrageenan, a new class of food additive derived from ***Eucheuma cottonii*** *and* ***E. spinosum*** *seaweeds, has been globally marketed since 1980. To prove that it is non-toxic and to earn the recognition of the Food Codex Alimentarius and the FAO/WHO Joint Expert Committee on Food Additive (JECFA) as a food additive and, thereby, eliminate future international trade barriers, several toxicological studies were carried out using the conventionally processed (CP) carrageenan as the positive control. A three-month subchronic toxicity test was carried out in rats to examine the adverse effects over a period of time and the cumulative toxicity on target organs and on physiologic and metabolic tolerance from repeated exposure. The following parameters were observed; general observation, body weight, feed efficiency, intake of test materials, clinical examinations (hematology, serum biochemistry, blood coagulation analysis, urinalysis, and fecalysis), and gross, light microscopic and TEM examinations of tissues and organs. A battery of genotoxicology tests, namely Rec assay to determine direct DNA damaging potential, Ames test to assess the mutagenic potential before metabolic activation, Host-mediated assay to determine the mutagenicity after metabolic activation, Micronucleus test to investigate the chromosome breaking effect as well as antigenotoxic activities were done. The subchronic toxicity test in rats revealed the following findings: 1. There were no toxic manifestations or adverse effects observed with PNG and CP carrageenan in rats at a dose that is 80 times more than the Acceptable Daily Intake (ADI) set by the FAO/WHO JECFA for CP carrageenan 2. There was no evidence that PNG and CP carrageenan absorbed from the GIT.*

The genotoxicity tests showed that PNG and CP carrageenan 1. do not possess any direct DNA damaging potential, 2. are not mutagenic before and after metabolic activation, 3. have no chromosome breaking effects, and 4. reduced the chromosome breaking effects of the three known genotoxins. From all the studies that were done, it can be concluded that PNG carrageenan has the same toxicological attributes as CP carrageenan and does not present any food safety concern. An ADI for PNG carrageenan, similar to CP carrageenan, can be assigned by the FAO/WHO JECFA. So far in the past years, the Philippine Government has made several significant success in its campaign for PNG carrageenan's international recognition as a food additive and they are as follows: 1. In 1995, the Food Codex Alimentarius, Codex Committee on Food Additives and Contaminants, granted PNG carrageenan a Code No. E 407a under the International Numbering System, 2. In 1996, the European Union lifted the ban on PNG carrageenan as a food additive and allowed its entry in the European Single Market. 3. In 1994, the FAO/WHO JECFA required additional data on PNG carrageenan and calendared it for another round of review in their 1998 meeting to assign a final ADI. Consequently, the new status of PNG carrageenan triggered significant growth in the carrageenan industry in terms of strengthening the economy of the Philippines, expansion of the carrageenan market, improvement of technology and employment security to the marginalized fisherfolks of the Southern and Central Philippines. The seaweed is now one of the nation's 14 top export industries. The industry's total export in 1996 reached US$120 million compared to only about US$16 million in 1990, 750% increase.

Keywords: *E407a, PNG, Carrageenan, Rats, Genotoxicity, Antigenotoxicity, Subchronic Toxicity, Food Additive, Seaweeds, Euchema.*

INTRODUCTION

Carrageenan is a mixture of highly sulfated polygalactosides extracted from seaweeds (Stoloff 1959). Carrageenan is further classified as degraded (polygeenan) and native (undegraded) carrageenan. Degraded carrageenan is prepared by mild hydrolysis of iota carrageenan resulting to low molecular substance of 20,000 to 30,000. It has no food value since it lacks gelling and thickening properties, but has medical value (Bonfils 1970; Di Rosa 1972) and toxicological importance (Marcus and Watt 1969). The native carrageenan is widely used as food additive for its gelling and thickening properties and has been evaluated for acceptable daily intake at 50 mg/kg body weight/day in man by the joint FAO/WHO Expert Committee on Food Additives in 1969 and 1974. As of 1984, however, the estimate of Acceptable Daily intake for man has not been specified by the same committee. The detailed structure varies slightly depending on the source, but there are three main types: kappa, which produces a stronger and more brittle gel; iota, which produces a weaker and elastic gel; and lambda, which has no gelling property but has value as thickener (Technical Dosier 1991).

Approximately 70 to 80% of carrageenan worldwide is being produced today from two main species of seaweeds, *Eucheuma cottonii* and *E. spinosum*, mainly because these two species have been successfully cultivated on large scale farms,

Eucheuma cottonii is mainly composed of kappa-carrageenan, while *E. spinosum* is mainly composed of iota carrageenan (Technical Dosier 1991).

Food grade carrageenan has been produced mainly in Europe and the United States for more than 30 years by subjecting certain red seaweeds to conventional refining process. In the mid-1970's, a new technology which requires low energy of inputs for refining eucheuma seaweeds was developed in Japan and later on adopted in the Philippines, where eucheuma seaweeds abound, as an alternative refining process. This process retains the carrageenan together with the cellulose after removing the impurities as opposed to the conventional process where the cellulose is removed with the impurities leaving only the carrageenan behind. Thus, PNG carrageenan's main difference from the CP carrageenan is the presence of higher percentage of cellulose, which mostly makes up the acid-insoluble matter, at 12% against less than 1%, and, correspondingly, lower inorganic salt contents at 10 to 15% against 25%. The molecular weight of pure carrageenans from both carrageenans is more than 100,000 and range as high as 800,000 (Technical Dosier 1991).

In the Philippines, carrageenan from eucheuma seaweeds, produced by the alternative refining process, is known as Philippine Natural Grade (PNG) carrageenan. It has also been known by other names. The toxic effects of orally administered carrageenan, mostly of degraded type, have been shown to cause ulcer, metaplastic changes and tumor formations in the large intestine of several species of animals (Watt and Marcus 1969-1970; Grasso 1973; Fabian 1973; Benitz 1973; Wakabayashi 1978; Oohashi 1981). However, orally administered native (undegraded) carrageenan for 13 to 39 weeks from different botanical sources in rats revealed no evidence of carrageenan storage in the body and subchronic and chronic toxicity (Abraham 1985; Nilson and Wagner 1959) administered native carrageenan in rats and mice up to 25% level in their diet for 2 years and failed to demonstrate any adverse biological effects. However, it was able to produce ulcerations in the large intestine of the guinea pig and rabbit, but failed to produce the same lesion in rat, hamster, squirrel, monkey and ferret (Grasso et. al, 1973).

In 1980, PNG carrageenan was marketed in other countries as a new class of food additive carrageenan. The Philippines emerged as heavy exporter of refined carrageenan that competed with the giant manufacturers in the US and Europe.

In 1982, the US Food and Drug Administration (USFDA) imposed a ban on PNG carrageenan. It declared it unsafe to the 250 million American consumers. The new US carrageenan specification restricted AIM to 2%. The new definition denied PNG carrageenan the classification and label "carrageenan." Moreover, the USFDA reported that 12% AIM, although made up of cellulose and minerals, is toxic to man.

The Seaweed Industry Association of the Philippines or SIAP submitted physicochemical and microbiological data to the USFDA through the Department of Trade and Industry (DTI) and the Department of Foreign Affairs (DFA). In 1990, the eight-year import ban was finally lifted by the US government.

The victory of the seaweed industry was negated, in that same year. The European Union (EU) imposed a ban on PNG carrageenan since the FCA-CCFAC does not list PNG carrageenan as food additive.

In 1992, a Memorandum of Agreement was made and entered into by the Central Visayas Regional Projects Office (CVRPO), the Regional Office of the Department of Agriculture (Reg. VII), the SIAP represented by Mr. Benson U. Dakay, and the Philippine Council for Health Research and Development Foundation (PCHRD), Department of Science and Technology.

The CVRPO provided the funds for the toxicological studies, while the PCHRD was responsible for contracting laboratory facilities and services of the best qualified Filipino scientists. The PCHRD tapped Dr. Quintin L. Kintanar, as the Project Leader of the PNG Carrageenan Toxicological Studies. He collaborated with Professor Clara Y. Lim-Sylianco to undertake the genotoxicity and antigenotoxicity studies. The research staff of the BFAD Experimental Animal House Section, Laboratory Services Division conducted the subchronic toxicity test. Dr. Helen A. Molina and Dr. Kiyoshi Imai conducted the pathological examinations.

The research project aims to prove that PNG carrageenan is non-toxic and present no food safety concern is non-toxic. The result will be used to earn recognition from the Food Codex Alimentarius and the FAO/WHO Joint Expert Committee on Food Additive (JECFA) as a food additive and, thereby, eliminate future international trade barriers.

After the completion of the studies, the Philippine government formed a mission composed of representatives from the DTI, DFA, BFAD-DOH and the SIAP. It presented the findings before the food additive experts at the FAC-CCFAC meeting in Brussels and the 44th Meeting of JECFA in Rome.

MATERIALS AND METHODS

Three-Month Toxicity Test of PNG Carrageenan in Dietary Administration to Rats

A. Test and Control Materials. Philippine Natural Grade carrageenan (PNG Carrageenan, Bengel 350, lot no. NS 960A), refined using the alternative process by Shemberg Marketing Corporation in Cebu City, Philippine, was used as the test material. Conventionally processed carrageenan (Carrageenan) composed of approximately 90% kappa and 10% lambda carrageenan (Gelcarin GP 812; Product Specification No. 303), manufactured last January 1992 by Marine Colloids Division, FMC Corporation, United States of America was used as the positive control. The standard laboratory powdered diet for rats manufactured by the Bureau of Food and Drugs was used as the vehicle control.

B. Animals. One hundred twenty 4-week old specific pathogen-free outbred Sprague-Dawley (SD) rats of both sexes, 60 each, were transferred from the barrier-system production area to the testing area of the experimental animal house. Test male and female rats, 50 each, were selected after the acclimatization period. At the onset of the treatment period, the male and female rats were 6 weeks old.

The rats were selected at random and distributed to each experimental group, such that all experimental groups have nearly the same average body weight. Each experimental group consisted of 10 males or 10 females.

The animals were individually housed in hanging-type cages with mesh wire floorings provided with trays. The animals were allowed free access to their diet.

The room temperature, relative humidity, dark/light hour were set at 22 to 26°C, except on last quarter of feeding when temperature reached 29°C for 5 days due to air-condition malfunction; 40 to 90% relative humidity and 12 hours dark/12 hours light (7:00 am lights noon/7:00 pm off), respectively.

C. Preparation and Administration. PNG and CP carrageenan were admixed with powdered laboratory diet in the feed mixer. The mixing level of each test material is shown as follows:

Dose Group	Weight of Vehicle (kg)	Material and Weight (name, kg)	Total kg
0.5% PNG carrageenan	99.5	PNG carrageenan, 0.5	100
1.5% PNG carrageenan	98.5	PNG carrageenan, 1.5	100
5.0% PNG carrageenan	95.5	PNG carrageenan, 5.0	100
5.0% CP carrageenan	95.0	CP carrageenan, 5.0	100

The diet was orally administered using an aluminum feed containers with perforated covers for three months. The groupings and dose level of materials were as follows:

Sex/No.	Group No.	Animal No.	Test Compound	Level
Male				
10	1	11-20	None	Not applicable
10	2	21-30	PNG carrageenan	0.5%
10	3	31-40	PNG carrageenan	1.5%
10	4	41-50	PNG carrageenan	5.0%
10	5	51-60	CP carrageenan	5.0%
Female				
10	1	111-120	None	Not applicable
10	2	121-130	PNG carrageenan	0.5%
10	3	131-140	PNG carrageenan	1.5%
10	4	141-150	PNG carrageenan	5.0%
10	5	151-160	CP carrageenan	5.0%

D. Observation and Examination

1. **General Observations.** All animals were observed daily for any clinical signs and behavioral changes, including the gross appearance and consistency of the stools.

2. **Body Weight.** The weight of each animal was measured at pretreatment period, at the start of the treatment, and once a week thereafter until the termination of feeding.

3. **Feed Consumption.** Feed consumed by each rat were measured twice a week.

4. **Efficiency of Feed Utilization.** To weekly feed efficiency (%) the following formula was used was:

$$\frac{\text{Body Weight Gain (g)}}{\text{Feed Consumed (g)}} \times 100$$

5. Intake of Test Materials. Individual intake of test materials (mg/kg/day) was computed based on the following formula:

$$\frac{\dfrac{[\,(\text{Feed Consumed (g)} \times 1000) \times (\text{Dose Level (\%)}/100)\,]}{\text{Body Weight (g)} / 1000}}{7 \text{ days}}$$

6. Clinical Examination

a. **Hematological Examination.** The following tests were conducted: white blood cell count, red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, and differential count.

b. **Blood Coagulation System Test.** The following tests were conducted: prothrombin and activated partial thromboplastin time.

c. **Blood Serum Biochemical Examinations.** The following tests were conducted using automated chemistry analyzer: sodium, potassium and chloride: inorganic phosphorus, albumin, A/G ration, SGOT, SGPT, cholinesterase, alkaline phosphatase, phospholipids, total cholesterol, triglycerides, blood urea nitrogen, total bilirubin, glucose.

d. **Urinalysis.** The following tests were conducted: 1. fresh urine-pH, glucose, ketone bodies, bilirubin, blood, urobilinogen, and 2. 24-hour urine-volume, turbidity, color and specific gravity.

The presence of kappa carrageenan with metachromatic test modified from the method of Beattie et al [5] with a detection limit of 10 ppm.

e. **Fecalysis.** Fecal samples were collected on the 6th and 13th week of feeding and tested for the presence of occult blood using the Guaiac method.

E. Pathology

1. **Necropsy.** All animals were sacrificed at the end of the administration period by exsanguination under light ether anesthesia. Full necropsy, which included examination of the external surface of the body, all orifices and the cranial, thoracic, abdominal and pelvic cavities and their contents, were done.

2. **Organ Weight.** The following organs were weighed:

Brain	Spleen
Pituitary Gland	Kidneys (left and right)
Submandibular Glands	Adrenal Glands (left and right)
Thymus	Testes (left and right)
Lungs	Epididymis (left and right)
Thyroid	Prostate Gland
Heart	Ovaries (left and right)
Liver	Uterus
Cecum (with and without contents)	

3. **Microscopic Examination.** Fixed tissues and organs were trimmed, processed (ETP-12V; Sakura), embedded in paraffin (TECS 4589, Sakura Miles) and cut into slices of 3 to 4 micron in thickness using sledge microtome (IVS-400; Sakura) and stained with hematoxylin and eosin.

The liver, spleen, mesenteric lymph node, and gastrointestinal tract of the group which were fed with 5.0% PNG and CP carrageenan, were stained with 0.05% toluidine blue and examined for the presence of metachromatic materials.

4. **Transmission Electron Microscopy.** Electron microscopic observation was performed on the liver and kidney from two males and two females in the vehicle control group and the 5.0% PNG carrageenan groups.

F. **Statistics.** The YUKMS Statistical Library 1, Version 4.1; Division Statistical Analysis, Yukms Corp., Tokyo, Japan: Copyright 1987, 1989, 1991, a computer program, was used to calculate statistics.

Mutagenicity, Clastogenicity and Antimutagenicity Potential of Carrageenan

The following battery of tests were conducted on PNG carrageenan as the test substance and CP carrageenan as the positive control:

Rec Assay was used to determine direct DNA damaging potential (Kada, T.K. et al.; 1980).

Ames Test, without -9 mix, was employed to assess the mutagenic potential before metabolic activation (Ames, B.N.; 1971).

Host-mediated Assay was used to study mutagenicity after metabolic activation (Moriya, M.; 1980).

Micronucleus Test was used to investigate the chromosome breaking effect as well as antigenotoxic activity (Schmid, W.; 1978)

Genotoxins dimethylhydrazine, dimethylnitrosamine and benzo(a)pyrene were used to induce mutagenicity.

The Swiss Webster mice were used in the Micronucleus test.
ANOVA was used in statistical evaluations.

RESULTS

Three-Month Toxicity Test of PNG Carrageenan in Dietary Administration to Rats

A. General Observations

There were no mortalities in all groups of either sex.

There were no clinical signs and behavioral changes related to the administration of PNG carrageenan and CP carrageenan, except for changes in the consistency and appearance of feces (Table 1; 1.A and 1.B). A few rats fed with PNG carrageenan, particularly at 5.0% dietary level developed soft formed stool sporadically starting on the middle third of administration. Big sized and fragmented stools were also occasionally observed. Beaded appearance of stools, which were not observed with CP carrageenan, were also noted.

All animals fed with CP carrageenan passed soft formed stools, which had an unusual pungent odor, frequently from the initiation up to the termination of feeding. Big sized and fragmented stools were also observed frequently from the middle period up to the end of feeding.

The findings on fecal consistency and appearance with PNG carrageenan-fed rats were definitely observed at a lower frequency and involved fewer rats than CP carrageenan-fed rats in either sex.

Body Weight. There were no significant differences in body weight among all groups in either sex.

Feed Consumption. There were no significant differences in feed consumption among all groups in either sex.

Efficiency of Feed Utilization. There were no significant differences in the efficiency of feed utilization among all groups in either sex.

Intake of Test Materials. The average daily intake of PNG carrageenan at different mixing levels were 382, 1140 and 3887 mg/kg/day in male rats and 410, 1292 and 4170 mg/kg/day in female rats. On the other hand, the average daily intake of CP carrageenan in male and female rats were 3917 and 4249 mg/kg/day, respectively (Table 2).

Average daily intake of PNG carrageenan at 0.5, 1.5 and 5.0% level of the diet were approximately 8, 25 and 80 times, respectively, more than the JECFA acceptable daily intake of 50mg/kg/day (1974) for an adult man.

Table 1. General and Fecal Observations
Table I.A. In Male Rats

DIETARY GROUP	OBSERVATIONS	No. of Weeks After Initiation of Feeding Frequency of Observation (No. of Rats Affected or Observed)													
		1	2	3	4	5	6	7	8	9	10	11	12	13	Total
	STOOL														0
Vehicle	Soft						4(2)							2(1)	6
Control	Big-Size													2(1)	2
	Fragmented														
	Wound on gums												2(1)	2(1)	2
	STOOL														
PNG (0.5%)	Soft		8(4)	1(1)							1(1)			1(1)	11
Carrageenan	Big-size			1(1)			1(1)								2
	Fragmented										4(2)	2(2)		7(3)	13
	STOOL														
PNG (1.5%)	Soft								1(1)					2(1)	3
Carrageenan	Big-size		2(1)			1(1)	1(1)	5(2)						8(4)	17
	Fragmented									3(2)	2(1)	2(1)		6(3)	13
	Chromodacryorrhea													6(1)	6
	STOOL														
PNG (5.0%)	Soft		10(6)			12(6)	3(1)	3(1)	10(4)	6(2)	4(2)	12(4)	9(5)	21(7)	90
Carrageenan	Big-size					8(4)	18(5)	13(2)	5(3)	13(5)	14(5)	2(2)	6(5)	17(6)	96
	Fragmented					4(2)	11(4)	12(4)	12(6)	14(5)	20(7)	19(5)	7(4)	13(4)	112
	Bead-Shape							7(4)	3(3)		2(1)				12
	STOOL														
CP (5.0%)	Soft		70(10)	102(10)	78(10)	85(10)	75(10)	72(10)	52(10)	39(10)	98(10)	78(10)	35(10)	68(10)	836
Carrageenan	Big-size					4(3)	10(5)	34(7)	25(9)	26(10)	22(10)	10(6)	13(7)	26(8)	170
	Fragmented					2(1)			25(10)	62(10)	16(10)	17(9)	40(10)	16(8)	178

Table 1.B. In Female Rats

DIETARY GROUP	OBSERVATIONS	No. of Weeks After Initiation of Feeding Frequency of Observation (No. of Rats Affected or Observed)													
		1	2	3	4	5	6	7	8	9	10	11	12	13	Total
	STOOL														
Vehicle Control	Soft		1(1)												1
	Big-Size							1(1)							1
	Fragmented													2(2)	2
	Wound on gums													1(1)	1
	STOOL														
PNG (0.5%) Carrageenan	Soft														0
	Big-size					1(1)									1
	Fragmented							3(3)							3
	STOOL														
PNG (1.5%) Carrageenan	Soft		1(1)	1(1)	1(1)	1(1)									4
	Big-size		12(6)					7(2)			2(2)				21
	Fragmented										6(1)		1(1)	2(1)	9
	STOOL														
PNG (5.0%) Carrageenan	Soft					2(1)	2(1)	2(1)	3(3)		2(1)	7(2)	4(3)	3(2)	25
	Big-size		5(2)			4(2)	6(3)			1(1)			4(2)		20
	Fragmented							1(1)	7(4)	6(3)	2(1)	8(4)	10(2)	2(1)	36
	Bead-Shape							8(5)		1(1)	1(1)				10
	STOOL														
CP (5.0%) Carrageenan	Soft	5(5)	26(10)	94(10)	62(10)	68(10)	80(10)	67(10)	48(10)	40(10)	63(10)	52(1)	30(1)	49(1)	684
	Big-size					1(1)	3(3)	17(8)	10(4)	10(4)	5(3)	11(7)	6(4)	7(3)	78
	Fragmented						3(3)	14(7)	19(8)	43(8)	21(9)	15(8)	43(9)	13(8)	171
	Swollen Gums					2(1)									2

Table 2. Daily Intake of Test Materials

Sex	Dietary Groups	No. of Animals	Weeks After Initiation of Administration Average													Daily Intake
			1	2	3	4	5	6	7	8	9	10	11	12	13	
Male	Vehicle Control	10	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PNG (0.5%)	10	744	604	518	433	394	359	332	313	296	268	271	225	209	302
	Carrageenan		18[b](9)	31	33	20	40(8)	15	18(9)	15	16	24	8	10	12	151
	PNG (1.5%)	10	2250	1785	1517	1269	1163	1079	962	896	918	867	822	666	621	1148
	Carrageenan		97	66(9)	49(9)	41	16(9)	44(9)	71(9)	37	36	56(9)	54	33	23	449
	PNG(5.0%)	10	7494	6125	5058	4182	4008	3750	3395	3149	3057	2968	2831	2290	2229	3887
	Carrageenan		236	272	210(9)	165(9)	210(9)	221(9)	133	198	412(8)	217	486(9)	142	220	1474
	CP (5.0%)	10	6982	6470	5389	4316	4017	3711	3407	3147	3047	2936	2899	2372	2222	3917
	Carrageenan		707	351	202(9)	233(8)	164(9)	149(8)	247(9)	181(9)	161	168(9)	534	182	273	1445
Female	Vehicle Control	10	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PNG (0.5%)	10	629	560	501	457	417	401	402	372	368	337	336	291	261	410
	Carrageenan		50	34	30	150	29	23	37(9)	18	15(9)	31	60	36	41	101
	PNG (1.5%)	10	2018	1765	1523	1389	1315	1321	1206	1190	1173	1885	1028	869	785	1282
	Carrageenan		169	120	66(8)	60(8)	51(7)	122(9)	64	79(8)	77	97	124	88	47	327
	PNG (5.0%)	10	6758	5700	5009	4561	4396	4289	4126	3802	3729	3441	3194	2689	2518	4170
	Carrageenan		470	159	260(9)	369(9)	294(9)	329(9)	706(9)	166	214	146	132	252	26	1133
	CP (5.0%)	10	6939	5886	5072	4718	4352	4156	4154	3835	3703	3500	3545	2855	2518	4249
	Carrageenan		1169	302	298	497	454(9)	342	293	321	357	343	473(9)	284	253	1154

a= mean
b=standard deviation

unit: mg/kg BWT/day

Nos. in ( ) indicate the No. of Animals out of 10 whose Daily Intake of Test Materials were computed Based on the Feed Consumption Record. If ( ) is not present. all 10 animals were computed.

B. Clinical Examination

Hematology. There were no significant differences in red blood cell count, white blood cell count, hematocrit, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, reticulocyte count (Retics), and differential count among all groups in either sex.

Blood Coagulation System. There were no significant differences in prothrombin time, activated partial thromboplastin time among all groups in either sex.

Blood Serum Biochemical Examinations. There were no significant differences in the total protein (TP), albumin, creatinine (Creat.), albumin and globulin ratio (A/G), alkaline phosphatase (ALP), cholinesterase (ChE), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic oxaloacetic transaminase (SGPT), phospholipid (P lipid), total cholesterol (T Chol.), triglycerides (Trigl), blood urea nitrogen (BUN), total bilirubin (T Bil), glucose (Gluc.,) inorganic phosphorus (in P), chloride (Cl), and potassium (K) among all groups in either sex (Table 3).

In female rats fed at dietary level of 0.5, 1.5 and 5.0% PNG carrageenan and CP carrageenan, the levels of serum sodium were significantly lower than the vehicle control and in male rats fed with CP carrageenan, the level of serum calcium was significantly higher than the vehicle control and the 5.0% PNG carrageenan group. In the absence of any abnormal findings related to serum sodium and calcium level abnormalities, the values were considered normal and of no toxicological importance.

Urinalysis. There were no significant differences in fresh urine pH, protein, ketone bodies, glucose, absence of occult blood, and urobilinogen observations and 24-hour urine volume specify gravity, color and turbidity observations (Table 4) among all groups in both sexes. However, three female rats fed with carrageenan were found positive for bilirubin, but no indications of intrahepatic or extrahepatic lesions were noted (Cornelius 1970). The concentration of this bile pigment in the urine is directly proportional to the degree of biliary obstruction whether intrahepatic or extrahepatic. Certain substances might have interfered with the semi-quantitative test.

Apparently, there was a tendency of urine volume to increase in animals administered with 5.0% PNG carrageenan with correspondingly lower specific gravity and pale urine color compared with the vehicle control group.

No carrageenan was detected in the urine excreted by all animals administered with PNG and CP carrageenan using the urine metachromasia test with the detection limit of 10 ppm.

Fecalysis. Fecal samples collected on the 6th and 13th of administration from all groups of either sex were negative for fecal occult blood.

Table 3. Results of Serum Biochemical Examination

Sex	Dietary Groups	No. of Animals	T. Prot g/dL	Albumin g/dL	Gluc. mg/dL	A/G	Creat. mg/dL	BUN mg/dL	T.Bill. mg/dL	ALP IU/L	ChE IU/L	AST IU/L	ALT IU/L	Pilipid mg/dL	T.Chol mg/dL	Trigl mg/dL	Ca mg/dL	In.P mg/dL	Na m	Cl mol	K /L
Male	Vehicle	10	6.22[a]	3.67	165.3	1.4	0.62	43.0	0.09	161.9	179	130	55.2	182	77.5	174	11.0	8.1	152	112	5.1
	Control		0.50[b]	0.21	30.1	0.1	0.04	4.2	0.05	46.0	43	22	11.6	38	15.1	71	1.8	1.7	11	7	0.5
	PNG (.5%)	10	6.42	3.73	200.9	1.3	0.63	39.4	0.10	168.8	254	143	93.6	184	76.9	197	12.0	8.8	155	114	5.6
	Carrageenan		0.80	0.42	50.6	0.1	0.08	8.9	0.05	41.5	191	29	63.4	36	10.3	130	2.7	2.2	19	15	0.9
	PNG (1.5%)	10	6.09	3.70	1717	1.4	0.67	44.6	0.09	183.2	201	141	69.1	183	80.1	164	11.2	8.8	157	117	5.3
	Carrageenan		0.66	0.41	22.2	0.1	0.08	6.3	0.04	50.1	49	36	32.6	20	11.3	40	1.8	1.5	15	10	0.4
	PNG (5.0%)	10	5.55	3.52	181.7	1.4	0.65	41.4	0.10	146.9	185	139	72.7	166	76.7	117	10.3	8.1	156	116	5.2
	Carrageenan		0.78	0.38	26.9	0.1	0.08	5.3	0.03	28.6	44	43	55.9	18	11.2	21	1.7	1.0	15	9	0.4
	CP(5.0%)	10	6.74	3.96	192.4	1.4	0.70	41.2	0.11	172.2	236	168	72.2	167	78.7	121	13.4	9.2	156	117	5.3
	Carrageenan		0.99	0.43	19.9	0.1	0.08	8.4	0.02	52.9	53	61	37.4	33	12.9	49	2.7	1.9	18	14	0.9
Female	Vehicle	10	6.63	4.14	159.9	1.6	0.65	43.4	0.08	127.1	1708	147	53.7	203	87.7	130	11.9	6.9	150	113	5.1
	Control		049	0.20	28.6	0.1	0.05	7.2	0.05	34.8	350	30	12.5	28	12.2	52	0.9	2.1	2	3	0.9
	PNG (0.5%)	10	6.29	3.89	145.6	1.6	0.64	39.2	0.08	121.2	1370	125	43.2	170	81.1	81	11.0	6.1	145	112	4.7
	Carrageenan		0.32	0.20	17.3	0.1	0.07	6.3	0.04	41.9	279	15	7.7	28	13.0	28	0.9	1.7	2*	2	0.4
	PNG (1.5%)	10	6.10	3.85	150.9	1.6	0.62	41.5	0.08	113.0	1573	134	46.4	184	89.6	99	11.1	6.4	146	112	4.7
	Carrageenan		0.31	0.16	11.2	0.1	0.04	5.5	0.04	50.4	429	25	8.7	26	14.6	40	0.8	1.6	1*	2	0.2
	PNG (5.0%)	10	6.26	3.92	159.3	1.6	0.64	39.7	0.06	145.0	1497	133	56.7	186	79.3	126	11.9	6.8	144	110	4.6
	Carrageenan		0.40	0.20	18.5	0.0	0.03	1.9	0.02	35.4	409	21	14.8	36	8.3	68	1.0	1.8	2*	2	0.4
	CP(5.0%	10	6.64	4.07	194.8	1.6	0.68	39.0	0.09	132.7	1655	196	83.9	178	82.8	95	12.0	7.7	144	111	5.3
	Carrageenan		0.65	0.36	118.3	0.1	0.05	5.2	0.03	35.1	494	193	105	42	17.0	48	1.6	2.8	2*	4	23

a = mean

b = standard deviation

– $p<0.05$ vs 5.0% PNG Carrageenan and Vehicle Control Groups

* – $p<0.05$ vs Vehicle Control Group

Table 4. Result of 24-Hour Urine Examination.

Sex	Dietary Groups	No. of Animals	Urine Volume mL	Values -	Specific Gravity 1.010 - 1.019	1.020 - 1.029	1.030 - 1.039	1.040 - 1.049	1.050 - 1.059	1.060 - 1.069	1.070 - 1.079	1.080 - 1.089	Color LY	Y	DY	Turbidity clear	slight	turbid
Male	Vehicle	10	10.6[a]	1.0385	0	3	4	0	2	1	0	0	1	5	4	1	2	7
	Control		4.7[b]	0.0130														
	PNG (.5%)	10	11.0	1.0478	1	1	1	3	1	2	0	1	2	3	5	3	3	4
	Carrageenan		9.8	0.0210														
	PNG (1.5%)	10	12.4	1.0383	0	3	3	3	0	0	1	0	2	7	1	4	1	5
	Carrageenan		6.4	0.0150														
	PNG (5.0%)	10	17.9	1.0274	2	3	4	1	0	0	0	0	4	4	2	2	0	8
	Carrageenan		7.8	0.0100														
	CP (5.0%)	10	13.5	1.0322	1	4	4	0	0	1	0	0	0	8	2	3	0	7
	Carrageenan		4.0	0.0120														
Female	Vehicle	10	10.7	1.0267	1	6	3	0	0	N.A.	N.A.	N.A.	2	7	1	3	0	7
	Control		3.8	0.0070														
	PNG (0.5%)	10	12.4	1.0231	3	5	2	0	0	N.A.	N.A.	N.A.	2	7	1	0	0	10
	Carrageenan		4.3	0.0080														
	PNG (1.5%)	10	10.0	1.0295	1	5	3	1	0	N.A.	N.A.	N.A	6	2	2	2	0	8
	Carrageenan		2.8	0.0100														
	PNG (5.0%)	10	14.3	1.0242	4	4	1	0	1	N.A.	N.A.	N.A.	5	4	1	4	0	6
	Carrageenan		6.2	0.0100														
	CP (5.0%)	10	8.9	1.0313	2	2	4	1	1	N.A.	N.A.	N.A.	2	3	5	5	0	5
	Carrageenan		4.8	0.0120														

Legend:

a = mean
b = standard deviation

LY - Light Yellow
Y - Yellow
DY - Dark Yellow

N.A. -Not Applicable

C. Pathology

Necropsy. No gross lesions were observed related to the administration of PNG carrageenan, as well as CP carrageenan, in either sex. The distention of colon may be due to the presence of big-sized stools. Prominent lymphoid tissues in the colon are not unusual in rats.

Organ Weight. There were no significant differences in the absolute (Tables 5; 5.A., 5.B. and relative organ weight among all groups in either sex. However, there is a tendency for the cecal content to be heavier in animals which were fed with 5.0% PNG carrageenan compared with the vehicle control group in either sex.

Light Microscopic Examination. There were no microscopic lesions as a result of the administration of PNG and CP carrageenan in all tissues and organs examined.

Toluidine blue staining of the liver, spleen, mesenteric lymph nodes, and the gastrointestinal tract revealed no deposition of metachromatic materials. Mast cells, which also stained with toluidine blue, were present in some tissues.

Transmission Electron Microscope. The deposits of PNG and CP carrageenan were not observed in the liver, kidney and colon of rats and no toxic changes were also noted in these organs

Mutagenicity, Clastogenicity and Antimutagenicity Potential of PNG Carrageenan

The results of the batteries of test are summarized in Tables 6, 7, 8, 9 and 10.

DISCUSSIONS

Three-Month Toxicity Test of PNG Carrageenan in Dietary Administration to Rats

The results of the three-month subchronic toxicity test in rats of either sex revealed no evidence that PNG carrageenan was absorbed from the gastrointestinal tract and no toxic manifestations or adverse effects were observed as a result of ingesting PNG carrageenan up to 5.0% level in the diet. The only effects observed were physiological adaptations to the orally administered test material consisting of soft consistency of formed feces, a tendency of urine volume to increase with corresponding decrease in urine specific gravity and pale urine color, and a tendency for the weight of the cecal content to increase. The same findings were observed with CP carrageenan.

It was reported that metachromatic materials in urine and in the reticuloendothelial cells of the spleen and liver of rodents were proofs of carrageenan absorption (Anderson and Soman 1966; Eagleton *et al.* 1969). In the present study, metachro-

Table 5. Absolute Organ Weight.

Table 5.A In Male Rats

Dietary Groups	Body Weight g	Brain g	Hypo-physis mg	Subman-dibular g	Thy-roid mg	Lungs g	Thy-mus g	Heart g	Liver g	Adrenals mg L	Adrenals mg R	Kidneys g L	Kidneys g R	Spleen g	Cecum g or-gan	Cecum g con-tent	Pros-tate g	Epidi-dymis (g) L	Epidi-dymis (g) R	Testes g L	Testes g R
Vehicle	459	2.30[a]	12	0.71	29	1.61	0.32	1.27	14.8	24	22	1.20	1.26	0.62	1.82	5.97	1.09	0.67	0.66	1.6	1.6
Control	34	2.28[b]	3	0.06	7	0.13	0.06	0.09	2.3	4	2	0.16	0.13	0.23	0.23	1.17	0.33	0.06	0.05	1	0
																				0.1	0.0
																				0	2
PNG (0.5%)	484	2.28	11	0.69	28	1.62	0.33	1.35	15.7	23	20	1.38	1.32	0.62	1.84	5.70	1.05	0.64	0.65	1.5	1.5
Carrageenan	38	0.12	2	0.06	8	0.15	0.06	0.10	1.8	5	5	0.09	0.13	0.29	0.24	1.03	0.25	0.06	0.05	9	8
																				0.1	0.1
																				4	4
PNG (1.5%)	480	2.36	12	0.75	30	1.56	0.32	1.33	15.1	22	20	1.20	1.26	0.68	1.72	5.95	1.21	0.66	0.68	1.6	1.6
Carrageenan	39	0.22	3	0.07	8	0.21	0.07	0.11	1.1	6	3	0.11	0.11	0.10	0.23	1.40	0.41	0.07	0.06	2	1
																				0.1	0.1
																				2	3
PNG (5.0%)	465	2.26	12	0.73	29	1.50	0.30	1.30	14.2	22	22	1.22	1.24	0.66	1.76	7.12	1.20	0.67	0.68	1.6	1.6
Carrageenan	39	0.10	4	0.06	7	0.19	0.05	0.08	1.2	3	2	0.09	0.09	0.07	0.25	1.54	0.33	0.07	0.06	3	3
																				0.0	0.1
																				7	2
CP (5.0%)	457	2.28	12	0.73	29	1.53	0.29	1.28	14.1	22	20	1.29	1.29	0.63	1.91	6.08	1.38	0.60	0.62	1.4	1.5
Carrageenan	37	0.13	3	0.06	7	0.16	0.07	0.10	1.4	4	5	0.11	0.12	0.09	0.26	1.47	0.24	0.11	0.11	8	7
																				0.3	0.0
																				4	8

a = mean

b = standard deviation

Table 5.B In Female Rats

Ditary Groups	Body Weight	Brain	Hypo-physis	Subman-dibular	Thy-roid	Lungs	Thy-mus	Heart	Liver	Adrenals mg		Kidneys		Spleen	Cecum g		Uterus	Ovary mg	
	g	g	mg	g	mg	g	g	g	g	L	R	L	R	g	organ	content	g	L	R
Vehicle	257	2.05[a]	13	0.45	20	1.15	0.2,	0.82	8.3	31	28	0.78	0.77	0.42	1.24	3.82	0.68	62	59
Control	20	0.06	6	0.03	2	0.23	0.06	0.07	0.7	5	4	0.07	0.07	0.04	0.14	0.64	0.18	10	6
PNG (0.5%)	250	2.10	14	0.47	20	1.08	0.23	0.78	7.8	30	26	0.77	0.74	0.41	1.19	3.76	0.58	58	60
Carrageenan	20	0.6	6	0.4	5	0.06	0.03	0.06	0.7	4	6	0.07	0.07	0.04	0.09	0.42	0.17	8	8
PNG (1.5%)	266	2.07	15	0.49	20	1.13	0.24	0.85	8.1	30	20	0.80	0.79	0.44	1.28	4.27	0.65	55	54
Carrageenan	35	0.05	6	0.04	4	0.11	0.04	0.09	1.2	3	3	0.10	0.12	0.06	0.18	1.11	0.19	7	9
PNG (5.0%)	266	2.07	13	0.49	22	1.16	0.27	0.81	79	28	25	0.72	0.73	0.45	1.31	4.67	0.63	57	53
Carrageenan	26	0.04	5	0.04	6	0.17	0.05	0.08	0.7	4	4	0.06	0.06	0.05	0.14	0.97	0.20	13	9
CP (5.0%)	251	2.05	14	0.48	19	1.11	0.24	0.79	7.9	29	27	0.77	0.76	0.45	1.28	4.32	0.70	55	60
Carrageenan	22	0.10	6	0.03	2	0.13	0.03	0.07	1.0	4	3	0.63	0.07	0.05	0.25	1.55	0.31	10	11

a = mean
b = standard deviation

matic materials were not observed in the liver, spleen, mesenteric lymph nodes, and intestines, as well as in the urine, of the test animals orally administered with PNG and CP carrageenan. Our findings were consistent with the subchronic and chronic oral toxicity tests results of Abraham et al, 1985, wherein native carrageenans from different botanical source composed of lambda kappa and iota, have no evidence of carrageenan storage in rats.

Administration of PNG and CP carrageenan did not result in intestinal ulceration. It was suggested that intestinal uptake and ulceration were related; the presence of intestinal ulcers may indicate absorption of carrageenan. They observed that the carrageenan is taken up and stored by lysosomes of the macrophages at the site of ulcerations (Abraham *et al.* 1947). Our histopathological and histochemical findings revealed no accumulation of macrophages in the lamina propia of the gastrointestinal tract and no metachromatic granules in the subepithelial tissues. In addition, fecal samples were negative for occult blood which indicated the absence of intestinal ulceration.

It was reported that there was an upper limit to the size of carrageenan molecules to be absorbed which ranged from 10,000 to 85,000 (Pittman *et al.* 1976). In the present study, the molecular weight of PNG and CP carrageenan were high, more than 150,000 and up to 800,000, and may be the principal reason why there were no carrageenan absorbed.

The digestion of carrageenan in the gastrointestinal tract of rat was not determined in this study. However, it was reported that when carrageenan was fed in rat at dietary level of 2-20%, it was 90-100% excreted in the feces undigested, and consequently cannot have any direct nutritional value (Hawkins and Yaphe 1965). Analysis of fecal samples of mammals, including rat, fed with a variety of lambda, kappa and iota carrageenan, by gel electrophoresis and showed that degradation of high molecular weight carrageenan had occurred, either in the gut or in the feces (Pittman *et al.* 1976). In our study, the degradation of PNG or CP carrageenan was not known. The absence of any evidence of absorption and ulceration indicated that, if ever there was any degradation, the carrageenans were not degraded to as low as 85,000.

The absence of abnormal clinical signs and behavioral changes, histopathological alterations, and clinical examinations, consisting of hematology, serum biochemistry, coagulation test, urinalysis and fecalysis in rats indicated that there were no functional and morphological manifestations of toxicity or adverse effects related to PNG carrageenan, as well as CP carrageenan administration. Our findings in rat were consistent with other reports (Abraham et al. 1985; Nilson *et al. 1959*).

The principal effect induced by orally administered PNG and CP carrageenan in rats was the passage of soft stool. Bead-shaped appearance of feces were also occasionally observed with PNG carrageenan. The consistency of the stool may be attributed to the hydrophylic or osmotic property of carrageenan (Leegwater 1974), a non-nutritive polysaccharide and the bulk of which passes through the gastrointestinal tract unabsorbed (Hawkins 1965; Dewar 1970), which resulted to laxative

effect (aperient). In a 56 days feeding and in a lifespan carcinogenicity study of native carrageenan in rats observed some diarrhea, marked chiefly by feces which were semi-solid in consistency, in addition to the soft stool (Grasso *et al.* 1973; Rustia *et al.* 1980).

The lower frequency and late onset of observation and fewer animals observed with soft stool consistency in the group fed with 5.0% PNG carrageenan compared with the group fed with 5.0% CP carrageenan may be partially attributed to the difference in the cellulose content. Both diets have about the same value in terms of macro nutrients, proteins and fats; micro nutrients, vitamins and minerals and palatability as shown in the tables of body weight, feed consumption and efficiency of feed utilization. Philippine Natural Grade carrageenan contains on the average 12% cellulose, an inert substance and a source of dietary or crude fiber which escapes digestions (Anderson, 1988; (WHO Food Additives Series No. 8, 1975) while CP carrageenan contains less than 1% cellulose. When PNG carrageenan was admixed with the standard laboratory diet with approximately 4% crude fiber, the total dietary fiber increased to about 4.4%; and conversely, when CP carrageenan was admixed with the standard laboratory diet, dietary fiber correspondingly decreased to about 3.8%. The difference in the total intake of dietary fiber was about 12.5%. In the present study, there was a tendency for the weight of the cecal content to increase and beaded appearance of stools were observed with 5.0% PNG carrageenan, which may be due to the higher dietary fiber and correspondingly higher water content. Thus, the differences in the frequency, onset the number of animals affected may be due to the difference in cellulose content between the two diets.

The tendency of the urine volume to increase with the corresponding lower urine specific gravity and pale urine color in the absence of renal abnormalities, such as the inability of the kidney to concentrate urine, indicated slight increase in water intake (Coles, 1980). It was observed that an increase in water retention due to an increase in the amount of osmotically active substances from dietary components which are not completely digested and/or absorbed in the intestine resulted to a tendency of the animals to drink more (Leegwater, 1974).

The fact that neither functional nor morphologic alteration in all tissues and organs were observed with PNG carrageenan compared with the CP carrageenan is of particular interest, since the animals were exposed to daily oral dose for 90 days up to 80 times the magnitude of the acceptable daily intake (50 mg/kg; 1974) in adult man. The findings suggested that PNG and CP carrageenan have the same toxicological attributes. Furthermore, the high cellulose content of PNG carrageenan may have contributed to the improvement of the consistency of feces excreted.

B. Mutagenicity, Clastogenicity and Antimutagenicity Potential of PNG Carrageenan

The following were found true for both PNG and CP carrageenan:

Carrageenan does not possess direct DNA damaging potential (Table 6). The data on Rec assay showed that there was no inhibition zone that was observed

with carrageenan test samples. The Rec (-) organism, a mutant of *Bacillus subtilis,* does not have the recombination repair system, while the Rec (+) organism has the recombination repair system.

Mutagenicity before metabolic activation of *S. typhimurium* TA 100 was not observed (Table 7).

The data on the Host-mediated assay suggest that carrageenan is not mutagenic after metabolic activation (Table 8). The mutation frequency of the indicator organism which was injected into the peritoneal cavity was not affected by carrageenan samples which were given by oral gavage. This indicates that

Table 6. Direct DNA Damaging Potential of Carrageenan Using Rec Assay

	ZONE OF INHIBITION	
	Rec (+)	Rec(–)
Carrageenan		
PNG 100 mg/ml	0+ /–00	0+ /–00
50 mg/ml	0+ /–00	0+ /–00
25 mg/ml	0+ /–00	0+ /–00
CP 28 mg/ml	0+ /–00	0+ /–00
Negative Control		
Distilled Water	0+ /–00	0+ /–00
Positive Control		
Quinoline	19.53 +/0.98	23.58 +/–0.93

Table 7. Mutagenic Potential Before Metabolic Activation of Carrageenan Using Ames Test Without S-9 Mix

	No. of Revertants per Plate S. typhimurium, TA 100
Carrageenan	
PNG, 100 mg/ml	13.36 +/– 4.36
50 mg/ml	13.36 +/–3.38
25 mg/ml	7.89 +/–1.12
CP, 28 mg/ml	10.58 +/–4.36
Negative Control, Distilled Water	18.21 +/–3.13
Positive Control, Quinoline	Too Numerous to Count (Very High)

metabolites of carrageenan did not interact with DNA of the indicator organism.

The data on the Micronucleus test revealed no appreciable amount of micronucleated polychromatic erythrocytes were formed in the bone marrow. This means that no chromosome breaking effects were exhibited by the carrageenan samples in bone marrow of the mice (Table 9).

When carrageenan was administered together with three known genotoxins in mice, the data on the Micronucleus test revealed that the chromosome breaking effects of the three genotoxins were reduced by carrageenan (Table 10). Since the

Table 8. Mutagenic Potential of Carrageenan After Metabolic Activation Using the Host-Mediated Assay

	Mutation Frequency of Indicator S. typhimurium His G 46
Carrageenan	
PNG, 2500 mg/kg body weight	2.09 +/–0.33
PNG, 1250 mg/kg body weight	2.05 +/–0.71
PNG, 625 mg/kg body weight	1.48 +/–0.89
CP, 700 mg/kg body weight	2.16 +/–0.39
Negative Control, Distilled Water	2.36 +/–0.42
Positive Control, Benzo(a)pyrene	11.86 +/–1.36

Table 9. Chromosome Breaking Effect of Carrageenan Using the Micronucleus Test

	No. of Micronucleated Polychromatic Erythyrocyte per thousand
Carrageenan	
PNG, 2500 mg/kg body weight	2.33 +/–0.89 (1)
PNG, 1250 mg/kg body weight	2.21 +/–0.49 (1)
PNG, 625 mg/kg body weight	2.06 +/–0.82 (1)
CP, 700 mg/kg body weight	1.86 +/–0.32 (1)
Negative Control, Distilled Water	1.92 +/–0.94
Positive Control, Benzo(a)pyrene	7.94 +/–0.96

Note: (1) No significant differences with Negative Control at 0.05 and 0.01 Probability.

Table 10. Antigenotoxicity of Carrageenan Against Dimethylhydrazine (DMH), Dimethylnitrosamine (DMN) and Benzo(a)pyrene (BP)

	No. of Micronucleated Polychromatic Erythrocytes per thousand		
	DMH 7.14+/−1.23	DMN 7.86+/−0.67	BP 7.30+/−0.71
Plus PNG Carrageenan			
250 mg/kg body weight	2.43+/−0.73 (2)	2.58+/−0.078 (3)	2.58+/−0.68 (4)
1250 mg/kg body weight	2.64+/−0.98 (2)	2.60+/−0.250 (3)	2.61+/−0.92 (4)
625 mg/kg body weight	2.59+/−0.76 (2)	2.38+/−0.095 (3)	2.66+/−0.83 (4)
Negative Control, Distilled Water	2.12+/−0.95	2.12+/−0.95	2.12+/−0.95

Note 2, 3, 4:
With Significant Difference with DMH, DMN and BP at 0.5 Probability.

three genotoxins are metabolized to alkylating agents of DNA, there is a possibility that carrageenan or its metabolite trap the alkylating species or carrageenan inhibits their metabolism to species reactive with DNA.

Carrageenan, PNG and CP, does not posses direct DNA damaging potential. It is not mutagenic before and after metabolic activation. No chromosome breaking effects were exhibited. However, carrageenan inhibited the genotoxic activity of three known carcinogens.

Inference

In the present studies, PNG carrageenan has been shown to be free from any subchronic and genetic toxicities and, on the contrary, possesses antigenotoxic activity against known mutacarcinogens. Unmistakably, PNG carrageenan, just like CP carrageenan, has no food safety concerns as a food additive and an ADI similar to CP carrageenan can be assigned.

Achievements and Benefits of the Research

In 1995, the FCA-CCFAC granted PNG carrageenan a classification of E 407a under the FCA International Numbering System. In October 1996, consistent with the new status, the EU through the 313 member European Parliament upheld the recommendation of the EU Council to reclassify PNG carrageenan as a safe food additive. The EU lifted the ban on PNG carrageenan and allowed its entry in the European Single Market, an indication of international acceptance.

In 1994, JECFA calendared PNG carrageenan for another round of review in 1998 to assign a final Acceptable Daily Intake, the theoretical level of PNG carrag-

eenan that can be ingested daily by a 70 Kg person for long period of time without any risk. The Seaweed Association of the Philippines has commissioned a British-based laboratory center to undertake additional tests on PNG carrageenan.

As expected, the success triggered significant growth and development in the seaweed industry in terms of strengthening the economy of the Philippines, expansion of the industry and its global market, technological improvement and employment security to the marginalized fisherfolks.

The seaweed industry is already one of the nation's 14 top export industry and it is still growing. Its total export in 1996 reached US$ 120 M compared to only about US$ 16 million in 1990, a 750% increase. PNG carrageenan has annual sale of about US$ 77 M. With the opening of the European market in 1996, the industry expects an additional US$ 100 to 200 M worth of PNG carrageenan export in the near future.

The Philippines has remained the No. 1 supplier of dried weeds in the world with US$ 18.7 M export. The 1996 figure shows that the export of its three main products, namely the dried seaweed, the PNG and refined carrageenan in expanding. PNG carrageenan grabbed 64.18% of the 1996 total sales, while dried seaweed and refined carrageenan followed at 16.46 and 19.36%, respectively.

In 1986, the Philippines was only exporting PNG carrageenan to Japan, Holland, Denmark, Canada, USA, West Germany and the United Kingdom. Today, the market has also included other European and non-EU countries in Western Europe, and South America, China, Australia, New Zealand, the Southeast Asian nations, Africa, and Israel, among others.

The seaweed farm sector has been the most benefited. Seaweed farmers, once marginalized as fisherfolks, have now discovered the value of seaweeds. An annual return anywhere from P104,382 to P125,000 per hectare of seaweed farm can be realized. Seaweeds is now considered more profitable than rice and coconut crops in the Southern and Central Philippines.

In 1990, there were only 75,000 Filipino families engaged in seaweed farming. In 1994, the figure rose to 95,050 families, of which 49,750 families are in Tawi-Tawi and Sulu.

REFERENCES

Abraham, R., Benitz, K.F., Mankes, R., and Rosenblum, I. (1995). Chronic and Subchronic Effects of Various Forms of Carrageenan in Ras. Ecotoxicol-Environ-Safety, pp. 173-83.

Abraham, R., Fabian, R.J., Golberg, L., and Coulton, F. (1974). Role of Lysosomes in Carrageenan-induced Cecal Ulceration. Gastroenterology, 67, 1169.

Ames, B.N. 1971. The Detection of Chemical Mutagens by Enteric Bacteria. Chemical Mutagens 1:267-282. Plenum Press.

Anderson, J.A. and Bridges, S.R., (1988). Dietary Fiber Content of Selected Foods, 47 American Journal of Clinical Nutrition (Annex 4), pp. 440-7.

Anderson, W. and Soman, P.D. (1966). The Absorption of Carrageenans. J. Pharm. 18, 825-827.

Beattie, A., Blakemore W.R., Dewar E.T., and Warlick, M.H. (1970). A Study of Orally-Administered Degraded Carrageenan in the Baboon. Food Cosmet. Toxicol. 8, 257-266.

Benitz, K.-F., L., and Coulston, F. (1973). Intestinal Effects of Carrageenan in the Rhesus Monkey *(Macaca mulatta)*. Food Cosmetic Toxicology, 11, 565.

Bonfils, S. (1970). Carrageenan and the Human Gut. Lancet ii, 414-415.

Coles, E.H. (1980). Veterinary Clinical Pathology, Third Edition, W.B. Saunders Company Philadelphia, London, Toronto, pp. 221-223.

Cornelius, C.E. (1970). Liver Function. In: Clinical Biochemistry of Domestic Animals, edited by Kaneko, J.J., and Cornelius, C.E. 2nd ed., New York: Academic Press, 1970.

Dewar, E.T. and Maddy, Morag L. (1970). Faecal Excretion of Degraded and Native Carrageenan by the Young Rat. J. Pharm. Pharmac. 22, 791.

Di Rosa, M. (1972). Biological Properties of Carrageenan. J. Pharm. Pharmacol., 24,89-02.

Eagleton G.B., Watt, J., and Marcus, R. (1969). Effect of Subcutaneously Degraded Carrageenan on the Production of Histaminic Induced Gastric and Duodenal Ulceration, J. Pharma. Pharmacol: 21, 123.

Fabian, R.J., Abraham, R., Coulston, F., and Golberg, L. (1973). Carrageenan-induced Squamous Metaplasia of the Rectal Mucosa in the rat. Gastroenterology, 65, 265.

Grasco, P., Sharratt, M. Carpanini, F.M.B. & Gangolli, S.D. (1973). Studies on Carrageenan and Large-bowel Ulceraion in Mammals. Food Cosmetic Toxicology, 11, 555.

Hawkins, W.W. and Yaphe, W. (1965). Carrageenan as a Dietary Constituent for the Rat: Faecal Excretion, Nitrogen Absorption, and Growth. Can. J. Biochem. 43, 479.

Kada, T. K., Hirano, and Y. Shirazu. 1980. Detection of Environmental Mutagens by Rec. Assay. Chemical Mutagens 6:149-73, Plenum Press, N.Y.

Klose, R.E., and Glicksman, M. (1968). "Handbook of Food Additives." (F.E. Furia, ed.), Chemical Rubber Co. Cleveland, Ohio, p. 313.

Lassiter, J.W. and Edwards, H.M. Jr. (1982). Animal Nutrition, Reston Publishing Company, Inc., Reston Virginia a. Prentice-Hall Company, p. 239.

Leegwater, D.C., de Groot, A.P., and van Kalmthout - Kuyper, M. (1974). The Aetiology at Caecal Enlargement in the Rat. Food Cosmetic Toxicology, 12, 687.

Marcus, R. & Watt, J. (1969). Seaweeds and Ulcerative Colitis in Laboratory Animals. Lancet ii, 489.

Moriya, M. 1980. Workshop Manual, Southeast Asian Conference on Environmental Mutagens, Carcinogens and Teratogens.

Nilson, H.W. and Wagner, J.A. (1959). Feeding Test with Carrageenan. Food Res., 24, 235-239.

Oohashi, Y., Ishioka, T., Wakabayashi, K., and Kuwabara, N. (1981). A Study on Carcinogenesis Induced by Degraded Carrageenan Arising from Squamous Metaplasia of the Rat colorectum. Cancer Letters, 1, 267.

Pittman, K.A., Golberg, L., and Coulston, F. (1976). Carrageenan: The Effect of Molecular Weight and PolymerType on Its Uptake, Excretion and Degradation in Animals, Food Cosmetic Toxicology Vol. 14, pp. 85-93. Pergamon Press 1976. Printed in Great Britain.

Rutia, M. Shubik, P., and Patil, K. (1980). Lifespan Carcinogenicity Tests with Native Carrageenan in Rats and Hamsters. Cancer Letters, 11, 1.

Schmid, W. 1978. The Micronucleus Test. Chemical Mutagens 4:31-52, Plenum Press.

Stoloff, L. (1959). Carrageenan. In Industrial Gums, Polysaccharides and Their Derivatives. Edited by R.L. Whistler and J.N. BeMiller. New York: Academe Press, p. 83.

Technical Dosier, Philippine Natural Grade Carrageenan, Submitted by the Government of the Republic of the Philippines, Before the Commission of the European Communities Scientific Committee for Food, Oct. 1, 1991, 53 pages plus attachments.

Wakabayashi, K., Inagaki, T., Fujimoto, Y., and Fukuda, Y. (1978). Induction by Degraded Carrageenan of Colorectal Tumors in Rats. Cancer Letters, 4, 171.

Watt, J and Marcus, R. (1969). Ulcerative Colitis in the Guinea-pig Caused by Seaweed Extract. J. Pharm. Pharmac., 21, 187S.

Watt, J. and Marcus, R. (1970). Ulcerative colitis in Rabbits Fed Degraded Carrageenan. J. Pharm. Pharmac. 22, 130.

WHO, (1975) Microcrystalline Cellulose, in Toxicological Evaluation of Some Food Colours, Thickening Agents, and Certain Other Substances, WHO Food Additives Series No. 8, (Annex 5) pp. 47-52.