Toxicity & Teratogenicity Studies in Avian Embryos-**Calcium Carrageenan**
FDA Contract #71-330 No Date

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CALCIUM CARRAGEENAN

TOXICITY and TERATOGENICITY STUDIES in AVIAN EMBRYOS

FDA CONTRACT #71-330

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STUDIES on the TOXICITY and TERATOGENICITY
of CALCIUM CARRAGEENAN in AVIAN EMBRYOS

SUMMARY and CONCLUSIONS

Three concentrations of HCl were employed as solvents for calcium carrageenan in these studies. Mortality data indicate levels of 140 mg/kg to be toxic when administered in the air cell at 0 hrs. Similar injections proved to be more toxic to 96 hr embryos using the air cell administration route. Dose levels of 22.5 mg/kg and above produced significant increases in mortality in this protocol. Calcium carrageenan was tolerated at both injection times using yolk administration. These data indicate that calcium carrageenan was embryo-toxic.

Teratogenic evaluations of calcium carrageenan suggest that this material produced statistically significant increases in the incidence of abnormal embryos following yolk administration at 96 hr incubation. Either 0 or 96 hr injection times employing yolk injections yielded significant linear relationship between log dose and probit of abnormality incidence.

GENERAL PROCEDURES

The protocols as specified under FDA Contract #71-330 were followed in the investigation of toxicity and potential teratogenicity of the specified substance. The toxicity of the substance was evaluated from the percentage hatch of embryos injected either in the air cell or yolk at either zero hours (pre-incubation) or after 96 hours incubation to provide four separate evaluations.

EGG SOURCE AND HANDLING

All eggs used in these investigations were from Shaver Starcross pullets housed at the Poultry Research Center of the University of Arizona in Tucson. The parent stock was maintained on the University of Arizona breeder diet which had been formulated to provide more than adequate amounts of all the known nutrients required by the breeding hen.

The feed was specially prepared to assure no contaminations and did not contain any additive drugs such as antibiotics. All eggs prior to use (within 48 hours of lay) were candled to remove any containing blood spots, abnormal air cells or abnormal shells, and only clean eggs ranging in weight from 23 - 26 ounces per dozen were used.

The supply flock was tested to assure the absence of Pullorum and Mycoplasma gallisepticum.

The eggs were incubated in forced draft Jamesway 252 machines with automatic temperature and humidity controls and an automatic turning device.

COMPOUND HANDLING FOR INJECTION

The substance tested was solubilized in a number of the prescribed solvents in order to determine the maximum concentrations which could be employed. Where possible, water was the solvent of choice. Maximum

injection volume was 0.05 ml. and all solvents and glassware were autoclaved prior to preparation of the solutions for use. The dose levels were administered with a microliter syringe using sterilized needles.

The preliminary range-finding studies using each of the administration routes and times were carried out with 10 - 25 eggs per dose level and included solvent controls, untreated controls and either drilled or pierced controls.

The actual dose-response protocol was carried out in two or more injections on different days to produce a minimum of 100 eggs at each dose level in five or more levels selected from the range- finding studies.

EXAMINATIONS OF EMBRYOS AND CHICKS

Eggs were candled daily and the dead embryos removed, examined and any abnormalities recorded. Five chicks from each dose level in each hatch were X-rayed to determine any skeletal abnormalities. Additional eggs injected at the approximate LD-50 level and an additional level below that were incubated and embryos at 8, 14, 17 days and hatch chicks removed for histopathological examinations.

In additional studies representative chicks from the dose-response protocol were saved. These chicks were housed in electrically-heated battery brooders with raised wire floors and fed University of Arizona diets. Feed consumption and growth rates were evaluated at 6 weeks of age and a sample of the birds sacrificed for gross and histopathological examinations.

The remaining birds in each group were maintained to 6 months of age and then sacrificed.

DATA HANDLING

All data were coded on forms provided by FDA for computer input. In addition to summaries of mortalities and abnormalities, a number of statistical evaluations were carried out. These statistical analyses included the following for both mortality and the incidence of abnormal embryos:

1. Chi-square tests for all dose levels and for each level against the solvent control.
2. Linear regression analyses + chi square test of linearity.
   a. % response against dose
   b. % response against log dose
   c. log % response against dose
   d. arcsin transformation against dose
   e. arcsin transformation against log dose
3. Log dose against Probit using Finney's maximum likelihood method.
   a. Where significant, the LD-30, 50, 70 and 90's were estimated with 95% confidence intervals.
4. One-way analyses of variance.
5. Linear regression with replication.

Three concentrations of HCl were employed to solublize calcium carrageenan (71-05) for use in the test protocols. The dose levels employed are shown in Table 1. The 0.6N HCl solvent was used only in preliminary studies.

MORTALITY

The data, with respect to embryo mortality, for the different solvent concentrations and test protocols are shown in Tables 2 - 8. Yolk administrations of calcium carrageenan in 0.12N HCl failed to cause a significant increase in mortality at all dose levels with the exception of yolk administration at 96 hours employing a dose of 30 mg/kg (Tables 3 & 9). A comparison of all dose levels for the yolk-96 hour series with the solvent controls resulted in a significant chi-square value indicating calcium carrageenan to be embryo-toxic (Table 9).

Employing the air cell administration route and 0.24N HCl as the solvent vehicle, levels of 140 mg/kg resulted in significant ($P < 0.01$) increases in mortality. When 96-hour embryos were injected with calcium carrageenan in the air cell, the 75 mg/kg dose and below resulted in significantly less mortality, while levels of 112.5 mg/kg and above significantly increased mortality (Tables 4, 5 & 10). The 0.24N HCl solvent injected groups (air cell-96 hours) showed a very high mortality of 80.24% (Table 5). Levels of calcium carrageenan in the same solvent exhibited mortality ranging from 14 - 60% at levels below 75 mg/kg; comparisons of the calcium carrageenen-injected groups with the drilled or untreated controls showed statistically significant increases in mortality at levels of 22.5 mg/kg and above, while a level of 112.5 mg/kg resulted in 97.9% embryo deaths (Table 5).

Yolk administration of calcium carrageenan in 0.24N HCl did not significantly increase embryo mortality at either injection time (Table 10).

Air cell injections of calcium carrageenan at 0 hr in 0.6N HCl resulted in statistically significant increases in mortality for only the 0.2 mg/kg dose level (Tables 8 & 11). Additional statistical evaluations employing linear regression analyses of log dose vs probit of mortality indicated a significant linear relationship for only the air cell-96 hr series (Table 12). The LD-50 estimate from these calculations was 136.1 mg/kg. The remaining evaluations were non-statistically significant.

TERATOLOGY

Data on the occurrence of abnormal embryos and H-S-V-L abnormalities are shown in Tables 2 - 8. Chi-square analyses of these data indicated a significance ($P<0.05$) increase in abnormal embryos for the 30 and 37.5 mg/kg dose levels in the air cell-96 hr series (Table 14). Estimates of teratogenic dosages from probit analyses are shown in Table 16.

The 37.5 mg/kg dose injected in the air cell of 96 hr embryonated eggs significantly increased H-S-V-L abnormalities in comparison with the solvent control, while other dose levels in this series proved to be insignificant (Tables 17 - 19). The specific teratogenic findings are listed in Table 20. The evaluation of calcium carrageenan for teratogenesis suggests that this compound was teratogenic in the test protocols employed.

POST HATCH DATA

In those chicks maintained for six months post administration of calcium carrageenan, no significant differences in body weight at the three time periods evaluated or in feed consumptions were noted. Sexual maturity was slightly delayed for the 10 and 4 mg/kg levels; however, larger doses of 25 or 50 mg/kg did not result in a delay in sexual maturity in females.

TABLE 1

DOSE and SOLVENT SCHEDULES
for CALCIUM CARRAGEENAN

	Dose Range, mg/kg		
	0.12N HCl	0.24N HCl	0.6N HCl
Air Cell			
0 hrs	-	30 - 150	0.02 - 10.0
96 hrs	-	15 - 150	-
Yolk			
0 hrs	30 - 150	30 - 150	-
96 hrs	15 - 75	15 - 150	-

TABLE 2

CALCIUM CARRAGEENAN in 0.12N HCl
YOLK - 0 HRS

Dose, ppm	No. Fertile	Mortality %	Mortality #	Abnormal Total %	Abnormal Total #	Abnormal H-S-V-L %	Abnormal H-S-V-L #	Abnormalities by category: Head %	Head #	Skeletal %	Skeletal #	Viscera %	Viscera #	Limbs %	Limbs #	Structural %	Structural #	Toxic Response %	Toxic Response #	Functio %
150.0	93	26.88	25	2.15	2	2.15	2					1.07	1	1.07	1	2.15	2			
75.0	97	30.92	30	2.06	2	0.00	0									2.06	2			
60.0	99	24.24	24	2.02	2	3.03	3	2.02	2			1.01	1							
45.0	98	26.53	26	1.02	1	0.00	0									1.02	1			
30.0	94	36.17	34	1.06	1	1.06	1	1.06	1											
0.0	481	26.81	129	1.03	5	0.41	2	0.41	2							0.41	2			0.20
pierced	206	45.63	94	0.00	0	0.00	0													
ntreated	494	6.68	33	0.60	3	0.60	3	0.20	1			0.40	2							

SUMMARY - ALL DOSE LEVELS

481	28.90	139	1.66	8	1.25	6	0.62	3		0.42	2	0.21	1	1.04	5		

TABLE 3

CALCIUM CARRAGEENAN in 0.12N HCl
YOLK - 96 HRS

ose, ppm	No. Fertile	Mortality %	Mortality #	Abnormal Total %	Abnormal Total #	Abnormal H-S-V-L %	Abnormal H-S-V-L #	Abnormalities by category: Head %	Head #	Skeletal %	Skeletal #	Viscera %	Viscera #	Limbs %	Limbs #	Structural %	Structural #	Toxic Response %	Toxic Response #	Functional %	Functional #
5.0	108	19.44	21	1.85	2	0.92	1					0.92	1					0.92	1		
0.0	69	27.53	19	1.44	1	0.00	0									1.44	1				
5.0	70	25.71	18	1.42	1	1.42	1							1.42	1						
0.0	69	40.57	28	0.00	0	0.00	0														
5.0	67	19.40	13	0.00	0	0.00	0														
0.0	329	20.36	67	0.60	2	0.30	1	0.30	1											0.30	1
rced	156	30.76	48	0.64	1	0.64	1	0.64	1												
eated	494	6.68	33	0.60	3	0.60	3	0.20	1			0.40	2								

SUMMARY - ALL DOSE LEVELS

	383	25.85	99	1.04	4	0.52	2					0.26	1	0.26	1	0.26	1	0.26	1	

TABLE 4

CALCIUM CARRAGEENAN in 0.24N HCl
AIR CELL - 0 HRS

Dose, ppm	No. Fertile	Mortality %	Mortality #	Abnormal Total %	Abnormal Total #	Abnormal H-S-V-L %	Abnormal H-S-V-L #	Abnormalities by category: Head %	Head #	Skeletal %	Skeletal #	Viscera %	Viscera #	Limbs %	Limbs #	Structural %	Structural #	Toxic Response %	Toxic Response #	Function %
150.0	108	25.00	27	3.70	4	3.70	4	3.70	4											
140.0	34	76.47	26	5.88	2	2.94	1	2.94	1											
75.0	109	13.76	15	3.66	4	1.83	2	1.83	2											
60.0	108	15.74	17	3.70	4	1.85	2	1.85	2											
45.0	109	11.92	13	1.83	2	1.83	2	1.83	2											
30.0	110	7.27	8	0.90	1	0.90	1	0.90	1											
0.0	128	14.06	18	0.78	1	0.00	0													0.78
rilled	112	9.82	11	2.67	3	2.67	2	1.78	2					0.89	1					1.78
treated	494	6.68	33	0.60	3	0.60	3	0.20	1			0.40	2							

SUMMARY - ALL DOSE LEVELS

	578	18.34	106	2.94	17	2.08	12	2.08	12											

TABLE 5

CALCIUM CARRAGEENAN in 0.24N HCl
AIR CELL - 96 HRS

Dose, ppm	No. Fertile	Mortality %	Mortality #	Abnormal Total %	Abnormal Total #	Abnormal H-S-V-L %	Abnormal H-S-V-L #	Abnormalities by category: Head %	Head #	Skeletal %	Skeletal #	Viscera %	Viscera #	Limbs %	Limbs #	Structural %	Structural #	Toxic Response %	Toxic Response #	Functiona %	Functiona #
50.0	128	91.40	117	1.56	2	1.56	2			0.78	1			0.78	1	0.78	1				
12.5	94	97.87	92	1.06	1	0.00	0									1.06	1				
75.0	254	51.96	132	1.57	4	1.57	4	0.39	1			0.39	1	0.78	2					0.39	
60.0	149	32.21	48	3.35	5	3.35	5	0.67	1			0.67	1	2.01	3			0.67	1		
45.0	148	30.40	45	1.35	2	0.67	1							0.67	1			0.67	1		
37.5	106	63.20	67	4.71	5	4.71	5	3.77	4			0.94	1								
30.0	195	18.97	37	4.10	8	2.56	5	1.53	3			0.51	1	0.51	1	1.02	2	1.02	2		
22.5	108	43.51	47	1.85	2	2.77	3			0.92	1			1.85	2	0.92	1				
15.0	99	14.14	14	1.01	1	1.01	1					1.01	1								
0.0	486	80.24	390	1.23	6	1.44	7	0.61	3			0.20	1	0.61	3			0.20	1		
111ed	200	5.50	11	0.00	0	0.00	0														
reated	494	6.68	33	0.60	3	0.60	3	0.20	1			0.40	2								
SUMMARY - ALL DOSE LEVELS																					
	1281	46.76	599	2.34	30	2.03	26	0.70	9	0.16	2	0.39	5	0.78	10	0.39	5	0.31	4	0.08	

TABLE 6

CALCIUM CARRAGEENAN in 0.24N HCl
YOLK - 0 HRS

Dose, ppm	No. Fertile	Mortality %	Mortality #	Abnormal Total %	Abnormal Total #	Abnormal H-S-V-L %	Abnormal H-S-V-L #	Abnormalities by category: Head %	Head #	Skeletal %	Skeletal #	Viscera %	Viscera #	Limbs %	Limbs #	Structural %	Structural #	Toxic Response %	Toxic Response #	Functional %	Functional #
00.0	66	40.00	24	1.66	1	3.33	2	1.66	1					1.66	1						
50.0	48	6.25	3	0.00	0	0.00	0														
75.0	110	24.54	27	0.90	1	0.90	1	0.90	1												
50.0	104	13.46	14	0.00	0	0.00	0														
5.0	108	12.96	14	0.92	1	0.00	0									0.92	1				
0.0	110	17.27	19	0.00	0	0.00	0														
0.0	305	41.63	127	0.32	1	0.32	1	0.32	1												
rced	206	45.63	94	0.00	0	0.00	0														
eated	494	6.68	33	0.60	3	0.60	3	0.20	1			0.40	2								

SUMMARY - ALL DOSE LEVELS

540	18.70	101	0.55	3	0.55	3	0.37	2			0.19	1	0.19	1			

TABLE 7

CALCIUM CARRAGEENAN in 0.24N HCl
YOLK - 96 HRS

Dose, ppm	No. Fertile	Mortality %	Mortality #	Abnormal Total %	Abnormal Total #	Abnormal H-S-V-L %	Abnormal H-S-V-L #	Abnormalities by category: Head %	Head #	Skeletal %	Skeletal #	Viscera %	Viscera #	Limbs %	Limbs #	Structural %	Structural #	Toxic Response %	Toxic Response #	Functi %
150.0	30	10.00	3	6.66	2	6.66	2	6.66	2											
75.0	29	10.34	3	0.00	0	0.00	0													
60.0	68	19.11	13	0.00	0	0.00	0													
45.0	70	18.57	13	1.42	1	0.00	0									1.42	1			
30.0	70	24.28	17	0.00	0	0.00	0													
15.0	40	10.00	4	0.00	0	0.00	0													
0.0	95	17.89	17	0.00	0	0.00	0													
pierced	156	30.76	48	0.64	1	0.64	1	0.64	1											
untreated	494	6.68	33	0.60	3	0.60	3	0.20	1			0.40	2							

SUMMARY - ALL DOSE LEVELS

307	17.26	53	0.98	3	0.65	2	0.65	2				0.33	1

TABLE 8

CALCIUM CARRAGEENAN in 0.60N HCl
AIR CELL - 0 HRS

Dose, ppm	No. Fertile	Mortality %	Mortality #	Abnormal Total %	Abnormal Total #	Abnormal H-S-V-L %	Abnormal H-S-V-L #	Abnormalities by category: Head %	Head #	Skeletal %	Skeletal #	Viscera %	Viscera #	Limbs %	Limbs #	Structural %	Structural #	Toxic Response %	Toxic Response #	Functional %	Functional #
0.0	27	14.81	4	0.00	0	0.00	0														
4.0	27	25.92	7	3.70	1	3.70	1	3.70	1												
2.0	28	35.71	10	7.14	2	3.57	1	3.57	1							3.57	1				
0.2	28	46.42	13	10.71	3	14.28	4	7.14	2					7.14	2						
0.02	27	29.62	8	3.70	1	3.70	1	3.70	1												
0.0	28	17.85	5	0.00	0	0.00	0														
lled	112	9.82	11	2.67	3	2.67	3	1.78	2					0.89	1					1.78	2
eated	494	6.68	33	0.60	3	0.60	3	0.20	1			0.40	2								

SUMMARY - ALL DOSE LEVELS

	137	30.66	42	5.11	7	5.11	7	3.65	5			1.46	2	0.73	1			

TABLE 9

CALCIUM CARRAGEENAN in 0.12N HCl
CHI-SQUARE ANALYSES of MORTALITY

Dose Level mg/kg	Yolk	
	0 hrs	96 hrs
15.000	-	0.00
30.000	2.94	11.74*
45.000	0.00	0.69
60.000	0.16	1.33
75.000	0.49	0.01
150.000	0.01	-
All Doses (DF)	4.72 (5)	15.50* (5)

* Probability $<$ 0.05 - 0.005

TABLE 10

CALCIUM CARRAGEENAN in 0.24N HCl
CHI-SQAURE ANALYSES of MORTALITY

Dose Level mg/kg	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
15.00	-	165.13* (less)	-	0.80
22.50	-	59.43* (less)	-	-
30.00	2.15	220.79* (less)	19.99*(less)	0.65
37.50	-	13.40* (less)	-	-
45.00	0.09	128.56* (less)	27.91*(less)	0.01
60.00	0.03	120.71* (less)	26.03*(less)	0.00
75.00	0.02	62.84* (less)	9.40*(less)	0.46
112.50	-	16.19*	-	-
140.00	49.78*	-	-	-
150.00	3.86*	8.01*	20.83*(less)	0.55
300.00	-	-	0.01	-
All Doses (DF)	98.47* (6)	511.08* (9)	74.91* (6) (less)	6.20 (6)

* Probability < 0.05 - 0.005.

TABLE 12

CALCIUM CARRAGEENAN in 0.24N HCl
PROBIT ANALYSES - MORTALITY

	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
LD-30 (Range)	NS	82.9 (49.4 - 349.3)	NS	NS
LD-50 (Range)	NS	136.1 (83.2 - 4470.7)	NS	NS
LD-70 (Range)	NS	223.4 (116.7 - 68725.9)	NS	NS
LD-90 (Range)	NS	457.3 (179.5 - 3765804.0)	NS	NS

TABLE 13

CALCIUM CARRAGEENAN in 0.12N HCl
CHI-SQUARE ANALYSES of ABNORMALITIES

Dose Level mg/kg	Yolk	
	0 hrs	96 hrs
15.00	-	0.09
30.00	0.29	0.08
45.00	0.28	0.00
60.00	0.10	0.00
75.00	0.11	0.36
150.00	0.14	-
All Doses (DF)	1.64 (5)	3.28 (5)

TABLE 14

CALCIUM CARRAGEENAN in 0.24N HCl
CHI-SQUARE ANALYSES of ABNORMALITIES

Dose Level mg/kg	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
15.0	-	0.10	-	0.00
22.5	-	0.00	-	-
30.0	0.37	4.35*	0.28	0.00
37.5	-	4.04*	-	-
45.0	0.02	0.10	0.00	0.02
60.0	1.21	1.90	0.32	0.00
75.0	1.19	0.00	0.00	0.00
112.5	-	0.14	-	-
140.0	1.55	-	-	-
150.0	1.21	0.02	1.13	2.90
300.0	-	-	0.11	-
All Doses (DF)	6.26 (6)	12.60 (9)	4.11 (6)	16.91* (6)

* Probability $\leq$ 0.05 - 0.005.

TABLE 15

CALCIUM CARRAGEENAN in 0.60N HCl
CHI-SQUARE ANALYSES of ABNORMALITIES

Dose Level mg/kg	Air Cell 0 hrs
0.020	0.00
0.200	1.41
2.000	0.52
4.000	0.00
10.00	0.00
All Doses (DF)	5.94 (5)

TABLE 16

CALCIUM CARRAGEENAN in 0.24N HCl
PROBIT ANALYSES - ABNORMALITIES

	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
LD-30	NS	NS	211817.1	495.3
LD-50	NS	NS	1651991.0	892.2
LD-70	NS	NS	12883919.0	1606.9
LD-90	NS	NS	-	3758.2

TABLE 17

CALCIUM CARRAGEENAN in 0.12N HCl
CHI-SQUARE ANALYSES of HLSV ABNORMALITIES

Dose Level mg/kg	Yolk	
	0 hrs	96 hrs
15.0	-	0.78
30.0	0.00	0.75
45.0	0.09	0.08
60.0	1.19	0.75
75.0	0.10	0.00
150.0	0.00	-
All Doses (DF)	5.27 (5)	3.32 (5)

TABLE 18

CALCIUM CARRAGEENAN in 0.24N HCl
CHI-SQUARE ANALYSES OF HLSV ABNORMALITIES

Dose Level mg/kg	Air Cell 0 hrs	Air Cell 96 hrs	Yolk 0 hrs	Yolk 96 hrs
15.0	-	0.28	-	0.00
22.5	-	0.05	-	-
30.0	0.01	0.47	0.28	0.00
37.5	-	5.08*	-	-
45.0	0.68	0.01	0.30	0.00
60.0	0.70	1.21	0.32	0.00
75.0	0.68	0.08	0.00	0.00
112.5	-	0.14	-	-
140.0	0.51	-	-	-
150.0	2.86	0.00	1.13	2.90
300.0	-	-	0.11	-
All Doses (DF)	5.57 (6)	11.62 (9)	5.20 (6)	24.92* (6)

* Probability < 0.05 - 0.005.

TABLE 19

CALCIUM CARRAGEENAN in 0.60N HCl
CHI-SQUARE ANALYSES of HLSV ABNORMALITIES

Dose Level mg/kg	Air Cell 0 hrs
0.020	0.00
0.200	1.41
2.000	0.00
4.000	0.00
10.000	0.00
All Doses (DF)	6.08 (5)

Sheet 2

TABLE 20
CALCIUM CARRAGEENAN
TERATOGENIC FINDINGS

TREATMENT	TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
In 0.12N HCl - yolk - 0 hrs				
150.0 mg/kg	93	2	1	malrotation-foot, unilateral; agenesis-down; celosomia-abdomen
			1	dwarfism
75.0	97	2	1	agenesis-down
			1	dwarfism
60.0	99	2	1	anophthalmia-bilateral; celosomia-abdomen; brachy-gnathia-beak
			1	edema-neck
45.0	98	1	1	dwarfism
30.0	94	1	1	anophthalmia-bilateral; agenesis-beak
0.0	481	5	1	malposition
			1	curvature-beak; dysallilognathia-beak
			1	dysallilognathia-beak
			2	dwarfism

Sheet 3

TABLE 20
CALCIUM CARRAGEENAN
TERATOGENIC FINDINGS

TREATMENT	TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
In 0.12N HCl - yolk - 96 hrs				
75.0 mg/kg	108	2	1	celosomia - abdomen
			1	edema - head
60.0	69	1	1	dwarfism
45.0	70	1	1	malrotation-foot, unilateral
30.0	69	0	0	
15.0	67	0	0	
0.0	329	2	1	malposition
			1	anophthalmia-unilateral; dysallilognathia-beak
In 0.24N HCl - Air Cell - 0 hrs 150.0 mg/kg	108	4	1	anophthalmia-bilateral; dysallilognathia-beak
			1	microphthalmia-unilateral; encepholacele
			1	anophthalmia-unilateral; dysallilognathia-beak
			1	anophthalmia-bilateral; encephalocele; agenesis-be
140.0	34	2	1	anophthalmia-bilateral, vacuolization-liver
			1	vacuolization - liver
75.0	109	4	2	anophthalmia-unilateral; dysallilognathia-beak
			2	femoral contracture-bilateral

Sheet 4

TABLE 20
CALCIUM CARRAGEENAN
TERATOGENIC FINDINGS

TREATMENT	TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
In 0.24N HCl - Air Cell - 0 hrs				
60.0 mg/kg	109	4	1	anophthalmia-unilateral
			1	anophthalmia-unilateral; dysallilognathia-beak
			1	femoral contracture - bilateral
			1	femoral contracture - bilateral; femoral fracture-unilateral
45.0	109	2	1	anophthalmia-bilateral; encephalocele; shortened l
			1	anophthalmia-bilateral; agenesis-beak
30.0	110	1	1	anophthalmia-unilateral
0.0	128	1	1	ataxia
In. 0.24N HCl - Air Cell - 96 hrs				
150.0 mg/kg	128	2	1	agenesis-wing, unilateral; agenesis-neck
			1	dipygus
112.5	94	1	1	dwarfism
75.0	254	4	1	yolk sac absorption failure; agenesis-wing,unilate
			1	dysallilolagnathia-beak
			1	agenesis-leg, unilateral
			1	celosomia-abdomen

Sheet 5

TABLE 20
CALCIUM CARRAGEENAN
TERATOGENIC FINDINGS

TREATMENT	TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
In 0.24N HCl - Air Cell - 96 hrs				
60.0 mg/kg	149	5	1	edema-leg, unilateral
			1	agenesis-leg, unilateral
			1	hemorrhage-abdomen
			1	celosomia-eyelid, bilateral and abdomen; edema-
			1	contracture-toe, unilateral
45.0	148	2	1	agenesis-wing, unilateral
			1	edema-head
37.5	106	5	1	dysallilognathia-beak
			1	anophthalmia -unilateral; dysallilognathia-beak
			1	celosomia-abdomen
			2	microphthalmia-unilateral
30.0	195	8	1	ataxia
			1	anophthalmia-unilateral; dysallilognathia-beak
			1	acrania; agenesis
			1	dysallilognathia-beak; agenesis-wing, unilateral
			2	hypopigmentation-down
			1	celosomia-abdomen
			1	edema-head

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TABLE 20
CALCIUM CARRAGEENAN
TERATOGENIC FINDINGS

TREATMENT	TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
In 0.24N HCl - Air Cell - 96 hrs				
22.5 mg/kg	108	2	1	aplasia-wing, unilateral; curvature-vertebral co
			1	agenesis-wing, unilateral; dwarfism
15.0	99	1	1	celosomia-abdomen
0.0	486	6	1	malformation-wing, unilateral; curvature-leg, un
			1	hemorrhage-abdomen
			1	microphthalmia-unilateral; celosomia-abdomen; microphthalmia-unilateral; aplasia-wing, unilate
			1	aplasia-wing, unilateral
			1	anophthalmia-unilateral
In 0.24N HCl - Yolk - 0 hrs				
300.0 mg/kg	60	1	1	anophthalmia-unilateral; contracture-leg, bilater
150.0	48	0	0	
75.0	110	1	1	anophthalmia-bilateral
60.0	104	0	0	
45.0	108	1	1	aplasia-beak
30.0	110	0	0	
0.0	305	1	1	dicephalus

Sheet 7

CALCIUM CARRAGEENAN
TERATOGENIC FINDINGS

TREATMENT	TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
In 0.24N HCl - Yolk - 96 hrs				
150.0 mg/kg	30	2	1	agenesis-down
			1	encephalocele
75.0	29	0	0	
60.0	68	0	0	
45.0	70	1	1	dwarfism
30.0	70	0	0	
15.0	40	0	0	
0.0	95	0	0	
In 0.60N HCl - Air Cell - 0 hrs				
10.0 mg/kg	27	0	0	
4.0	27	1	1	cyclopia-head; agenesis-maxilla
2.0	28	2	1	hypopigmentation-down
			1	edema-neck
0.2	28	3	1	microphthalmia-bilateral
			1	curvature-leg, bilateral
			1	edema-head; contracture-toe, bilateral
0.02	27	1	1	anophthalmia-unilateral
0.0	28	0	0	

TABLE 21

CALCIUM CARRAGEENAN
POST HATCH DATA

Injection Date (10/11/71)

Label	Dose mg/kg	Age at Sexual Maturity, Days	Body Weight, gm					Average Feed Consumption	
			Hatch	6 weeks		6 months		6 wks	6 mos
				M	F	M	F	gm	kg
201	50.0	138	42.8	447	406	2007	1852	888	10.93
202	25.0	140	42.8	402	412	1703	1852	877	10.31
203	10.0	160	41.7	431	420	2043	1703	859	11.01
204	4.0	155	42.9	454	393	1930	1589	856	10.44
205	1.0	132	41.3	479	407	1930	1666	935	9.96
208	Solvent	141	41.4	478	423	1989	1788	975	11.18