

Propylene Glycol Alginate-Toxicity & Teratogenicity Studies in Avian Embryos-FDA  
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PROPYLENE GLYCOL ALGINATE

TOXICITY and TERATOGENICITY STUDIES  
in AVIAN EMBRYOS

FDA CONTRACT #71-330

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STUDIES on the TOXICITY and TERATOGENICITY  
of PROPYLENE GLYCOL ALGINATE in AVIAN EMBRYOS

SUMMARY and CONCLUSIONS

Propylene glycol alginate was not toxic to avian embryos at the levels tested in the four protocols. Statistical analyses of abnormalities observed failed to yield a significant chi-square value in any of the dose levels tested. The results of these tests suggest that propylene glycol alginate was neither embryo toxic nor teratogenic under the conditions of these tests.

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 (post-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.

Propylene glycol alginate (71-18) was solubilized in water for use in the test of protocols. The maximum dose level employed was obtained with a solution of 40 mg/ml to provide a dose of 40 mg/kg (2 mg/egg).

#### MORTALITY

The mortality data are shown in Tables 1 - 4. Propylene glycol alginate was relatively nontoxic in each of the 4 test protocols employed. Chi-square analyses of the mortality data, shown in Table 5, indicate a lack of statistical significance with the administration of this compound in comparison with water injected controls. Employing air cell after 96 hours incubation, dose levels of 10 and 20 mg/kg produced significantly less mortality than was obtained with the solvent control groups.

Probit analyses of log dose versus probit of mortality yielded a non-significant relationship (Table 6). These data suggest that propylene glycol alginate was relatively nontoxic to chicken embryos in the test protocols employed in this evaluation.

#### TERATOLOGY

The incidences of abnormal embryos in the fourth test protocols are shown in Tables - 1 4. The per cent abnormal embryos was quite low in all tests and ranged from 5 - 0%. Chi-square analyses of these data (Table 7) failed to indicate a significant increase in the incidence of abnormalities in comparison with the solvent control groups. Similar analyses of the head, limb, skeletal and visceral abnormalities did not yield significant chi-square values for any of the dose levels tested (Table 8). Specific teratogenic findings are listed in Table 9.

The results of these studies suggest that propylene glycol alginate was not teratogenic in any of the test protocols at the dose levels tested.

POST-HATCH DATA

Chicks which had received either two or twenty mg propylene glycol alginate per kg were fed to six months of age. The results (Table 10) failed to demonstrate any differences in body weights at either 6 weeks or 6 months of age and no significant differences in feed consumption or in age of sexual maturity were noted.



TABLE 2

PROPYLENE GLYCOL ALGINATE  
in WATER  
AIR CELL - 96 HRS

Dose, ppm	No. Fertile	Mortality % #		Abnormal				Abnormalities by category													
				Total		H-S-V-L		Head		Skeletal		Viscera		Limbs		Struc- tural		Toxic Response		Functional	
				%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#
20.0	165	1.21	2	0.00	0	0.00	0														
10.0	114	1.75	2	0.00	0	0.00	0														
5.0	120	5.83	7	1.66	2	1.66	2	1.66	2												
2.0	120	8.33	10	5.00	6	5.83	7	3.33	4		2.50	3		0.83	1						
1.0	115	6.08	7	2.60	3	2.60	3	2.60	3												
0.0	79	0.12	8	0.00	0	0.00	0														
drilled	89	7.86	7	0.00	0	0.00	0														
untreated	300	6.33	19	0.66	2	0.33	1				0.33	1		0.33	1						

## SUMMARY - ALL DOSE LEVELS

634	4.42	28	1.74	11	1.89	12	1.42	9		0.47	3		0.16	1					
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TABLE 3  
 PROPYLENE GLYCOL ALGINATE  
 in WATER  
 YOLK - 0 HRS

Dose, ppm	No. Fertile	Mortality % #		Abnormal				Abnormalities by category								
				Total		H-S-V-L		Head % #	Skeletal % #	Viscera % #	Limbs % #	Struc- tural % #	Toxic Response % #	Functional % #		
				%	#	%	#									
40.0	10	20.00	2	0.00	0	0.00	0									
20.0	108	18.51	20	0.00	0	0.00	0									
10.0	109	19.19	20	0.00	0	0.00	0									
5.0	104	28.84	30	0.96	1	0.96	1			0.96	1					
2.0	99	26.26	26	3.03	3	2.02	2	1.01	1	1.01	1			1.01	1	
1.0	99	56.56	56	0.00	0	0.00	0									
0.2	10	10.00	1	0.00	0	0.00	0									
0.0	97	17.52	17	1.03	1	1.03	1	1.03	1							
pierced	59	22.03	13	0.00	0	0.00	0									
untreated	300	6.33	19	0.66	2	0.33	1			0.33	1			0.33	1	

SUMMARY - ALL DOSE LEVELS

539	43.41	234	0.74	4	0.56	3	0.19	1		0.37	2			0.19	1	
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TABLE 4  
 PROPYLENE GLYCOL ALGINATE  
 in WATER  
 YOLK - 96 HRS

Dose, ppm	No. Fertile	Mortality		Abnormalities by category															
				Abnormal		Head		Skeletal		Viscera		Limbs		Structural		Toxic Response		Functional	
				%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#
20.0	120	33.33	40	0.83	1	1.66	2	0.83	1			0.83	1						
10.0	119	40.33	48	0.84	1	0.84	1					0.84	1						
5.0	118	39.83	47	1.69	2	2.54	3	0.84	1			0.84	1	0.84	1				
2.0	119	47.05	56	0.84	1	0.84	1					0.84	1						
1.0	119	38.65	46	1.68	2	1.68	2	0.84	1			0.84	1						
0.0	60	31.66	19	3.33	2	6.66	4	3.33	2			1.66	1	1.66	1				
pierced	60	28.33	17	1.66	1	0.00	0											1.66	1
untreated	300	6.33	19	0.66	2	0.33	1					0.33	1			0.33	1		

SUMMARY - ALL DOSE LEVELS

649	36.52	237	1.08	7	1.39	9	0.46	3			7.70	5	0.15	1					
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TABLE 5  
 PROPYLENE GLYCOL ALGINATE  
 CHI-SQUARE ANALYSES of MORTALITY

Dose Level mg/kg	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
0.200	0.047	-	0.026	-
1.000	0.703	0.359	1.53	0.567
2.000	0.043	0.008	1.703	3.275
5.000	0.000	0.454	2.986	0.813
10.000	0.048	4.075*(less)	0.028	0.937
20.000	0.027	7.022*(less)	0.000	0.003
40.000	0.162	-	0.057	-
All Doses (DF)	8.366(7)	13.981(5)	15.32(7)	6.347(5)

TABLE 6  
 PROPYLENE GLYCOL ALGINATE  
 PROBIT ANALYSES  
 MORTALITY

Air Cell		Yolk	
0 hrs	96 hrs	0 hrs	96 hrs
NS	NS	NS	NS

TABLE 7

PROPYLENE GLYCOL ALGINATE  
CHI-SQUARE ANALYSES of ABNORMALITIES

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Dose Level mg/kg	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
0.200	0.838	-	1.969	-
1.000	0.118	0.255	0.000	0.029
2.000	0.084	1.290	0.235	0.372
5.000	0.152	0.016	0.438	0.026
10.000	0.152	0.000	0.003	0.372
20.000	0.000	0.000	0.003	0.381
40.000	0.838	-	1.969	-
All Doses (DF)	4.798(7)	14.795(5)	9.170(7)	2.631(5)

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TABLE 8

PROPYLENE GLYCOL ALGINATE  
CHI-SQUARE ANALYSES of HLSV ABNORMALITIES

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Dose Level mg/kg	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
0.200	0.838	-	1.969	-
1.000	0.118	0.255	0.000	0.029
2.000	0.084	0.903	0.000	0.372
5.000	0.152	0.016	0.438	0.026
10.000	0.152	0.000	0.003	0.372
20.000	0.335	0.000	0.003	0.381
40.000	0.083	-	1.969	-
All Doses (DF)	7.602(7)	12.027(5)	5.628(7)	2.631(5)

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TABLE 9

Sheet 2

## TERATOGENIC FINDINGS

TREATMENT	TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS	
			NO.	D E S C R I P T I O N
Propylene Glycol Alginate in Water Air Cell - 0 hrs				
40.00 mg/kg	10	0	0	
20.0	153	1	1	granulation tissue - renal tubule
10.0	109	0	0	
5.0	109	0	0	
2.0	96	1	1	shortened - maxilla
1.0	100	0	0	
0.2	10	0	0	
0.0	50	1	1	thoracopagus
In Water - Air Cell - 96 hrs				
20.00 mg/kg	165	0	0	
10.0	114	0	0	
5.0	120	2	1	microphthalmia - rt.
			1	anophthalmia - bilateral, fusion failure - skull, agenesis-maxilla
2.0	120	6	1	anophthalmia-bilateral, dysgnathia, celosomia
			2	exencephaly

TABLE 9

Sheet 3

## TERRATOGENIC FINDINGS

TREATMENT	TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS	
			NO.	D E S C R I P T I O N
Propylene Glycol Alginate				
In Water - Air Cell - 96 hrs				
2.0 mg/kg cont'd			1	anophthalmia - bilateral, exencephaly, agenesia-maxilla, celosomia
			1	celosomia
			1	dwarfism
1.0	115	3	2	anophthalmia - rt., dysgnathia
			1	exencephaly, agenesia - maxilla
0.0	79	0	0	
In Water - Yolk - 0 hrs				
40.0 mg/kg	10	0	0	
20.0	108	0	0	
10.0	109	0	0	
5.0	104	1	1	celosomia
2.0	99	3	1	celosomia, dicephalus
			1	celosomia
			1	umbilical cord around fetus

TABLE 9

Sheet 4

TREATMENT	TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	TERATOGENIC FINDINGS	
			NO.	SPECIFIC FINDINGS
Propylene Glycol Alginate				
In Water - Yolk - 0 hrs cont'd				
1.0 mg/kg	99	0	0	
0.2	10	0	0	
0.0	97	1	1	microphthalmia - left, fusion failure-skull, dysgnathia
In Water - Yolk - 96 hrs				
20.0 mg/kg	120	1	1	anophthalmia - bilateral, microcephaly, brachygnathia- mandible, incomplete yolk absorption
10.0	119	1	1	celosomia
5.0	118	2	1	celosomia
			1	brachygnathia - mandible, agenesis - left wing, agenesis- left hindlimb
2.0	119	1	1	celosomia
1.0	119	2	1	anophthalmia - bilateral, shortened - maxilla
			1	celosomia
0.0	60	2	1	microcephaly - head, agenesis-bilateral wings, phocomelia - rt. hindlimb, agenesis - left hindlimb, celosomia
			1	anophthalmia - bilateral, exencephaly, shortened - maxilla

TABLE 10

## PROPYLENE GLYCOL ALGINATE

## POST HATCH DATA

Injection Date - 4/20/72

Label	Dose Level mg/kg	Age at Sexual Maturity	Average Body wt., gm.				Average Feed Consumption		
			At Hatch	6 wks		6 mos		6 wks	6 mos
				M	F	M	F	gm	kg
216	0.0	146	45.9	450	407	1816	1589	885	8.36
217	2.0	148	46.3	447	373	1703	1535	737	8.18
220	20.0	145	44.5	454	369	1703	1589	856	8.13