Toxicity & Teratogenicity Studies in Avian Embryos-**Calcium Silicate**-FDA Contract #72-345 **No Date**

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CALCIUM SILICATE

TOXICITY and TERATOGENICITY STUDIES in AVIAN EMBRYOS

FDA Contract #72-345

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STUDIES on the TOXICITY and TERATOGENICITY of CALCIUM SILICATE

SUMMARY and CONCLUSIONS

Calcium silicate, injected as a suspension in a glycerine-alcohol mixture, was found to be toxic to avian embryos under the conditions of these studies. Levels of 56 mg/kg and above significantly increased embryo mortality when administered in the air cell at 96 hrs. Yolk administrations of 28 mg/kg and higher doses were toxic following yolk administration at either 0 or 96 hrs.

Calcium silicate was not teratogenic in these studies.

GENERAL PROCEDURES

The protocols as specified under FDA Contract #72-345 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.

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.

Calcium silicate (71-41) was employed as a suspension in a glycerine-ethanol mixture in the four test protocols. The maximum dose level of 100 mg/kg was attained with a suspension of 100 mg of calcium silicate/ml.

MORTALITY

The mortality data obtained in the four test protocols with the injection of calcium silicate are shown in tables 1 - 4. Chi-square analyses of these data indicated that the injection of 56 mg/kg and higher levels into the air cell of 96 hour embryos significantly increased embryo mortality; while levels of up to 100 mg/kg, in the air cell of fertile eggs prior to incubation, did not significantly alter mortality patterns (Table 5). Yolk injections of calcium silicate at either 0 or 96 hrs were embryo toxic at levels of 28 mg/kg and above.

Linear regression analyses of log dose against probit of mortality were not statistically significant for any of the test protocols (Table 6).

TERATOLOGY

The occurrences of abnormal embryos and H-S-V-L abnormalities are shown in Tables 1 - 4. Chi-square analyses of the data on the occurrence of abnormal embryos failed to indicate a significant increase in the incidence of abnormalities as a result of calcium silicate injections in either the air cell or yolk. Probit analyses of these data were also not statistically significant at the 0.05 level of probability (Table 8).

Evaluations of H-S-V-L abnormalities do not suggest that dose levels up to 100 mg/kg resulted in a significant increase in the incidence of these in comparison with the solvent-injected control groups (Table 9).

The individual teratogenic findings are shown in Table 10.

Table 1

Calcium Silicate

in Glycerine: Ethanol

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 #
100.0	38	26.31	10	0.00	0	0.00	0														
70.0	133	25.56	34	1.50	2	0.75	1	0.75	1									0.75	1		
56.0	134	26.86	36	0.74	1	0.00	0									0.74	1				
28.0	135	20.74	28	2.96	4	3.70	5	1.48	2			1.48	2	0.74	1						
14.0	131	24.42	32	0.00	0	0.00	0														
7.0	127	29.13	37	0.78	1	0.78	1							0.78	1						
0.0	167	20.95	35	2.99	5	1.19	2	1.19	2									1.79	3		
drilled	150	8.66	13	0.00	0	0.00	0														
untreated	454	12.11	55	0.66	3	0.66	3	0.22	1			0.22	1	0.22	1			0.44	2		

SUMMARY - ALL DOSE LEVELS

698	25.36	177	1.15	8	1.00	7	0.43	3		0.29	2	0.29	2	0.14	1	0.14	1		

Table 2

Calcium Silicate

in Glycerine: Ethanol

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 #	Functional %	Functional #
100.0	38	44.73	17	0.00	0	0.00	0														
70.0	176	36.36	64	3.97	7	2.84	5	0.56	1			0.56	1	1.70	3	0.56	1	0.56	1		
56.0	180	42.77	77	4.44	8	4.44	8	0.55	1					3.88	7	0.55	1				
28.0	182	17.58	32	0.54	1	0.00	0									0.54	1				
14.0	180	9.44	17	0.55	1	1.11	2					0.55	1	0.55	1						
7.0	185	10.27	19	2.16	4	2.70	5	1.08	2			1.08	2	0.54	1	1.62	3				
0.0	218	25.22	55	3.21	7	3.66	8	1.37	3			0.91	2	1.37	3	0.45	1				
drilled	202	7.42	15	0.99	2	0.49	1					0.49	1					0.49	1		
untreated	454	12.11	55	0.66	3	0.66	3	0.22	1			0.22	1	0.22	1			0.44	2		

SUMMARY - ALL DOSE LEVELS

941	24.02	226	2.23	21	2.13	20	0.43	4		0.43	4	1.28	12	0.64	6	0.11	1	

Table 3

Calcium Silicate

in Glycerine: Ethanol

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 #
100.0	38	55.26	21	2.63	1	2.63	1	2.63	1												
70.0	145	51.72	75	3.44	5	3.44	5	0.68	1			2.75	4					0.68	1		
56.0	139	46.04	64	3.59	5	2.15	3					2.15	3			0.71	1	0.71	1		
28.0	140	55.00	77	1.42	2	0.71	1					0.71	1					0.71	1		
14.0	146	44.52	65	0.00	0	0.00	0														
7.0	127	45.66	58	1.57	2	1.57	2					1.57	2			0.78	1				
0.0	187	37.96	71	3.74	7	4.27	8	1.06	2			2.13	4	1.06	2						
pierced	60	26.66	16	3.33	2	0.00	0									1.66	1	1.66	1		
untreated	454	12.11	55	0.66	3	0.66	3	0.22	1			0.22	1	0.22	1			0.44	2		

SUMMARY - ALL DOSE LEVELS

735	48.98	360	2.04	15	1.63	12	0.27	2			1.36	10			0.27	2	0.41	3		

Table 4

Calcium Silicate

In Glycerine: Ethanol

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 #	Functional %	Functional #
100.0	38	52.63	20	0.00	0	0.00	0														
70.0	134	32.08	43	2.23	3	1.49	2	1.49	2							0.74	1				
56.0	133	44.36	59	3.75	5	1.50	2	1.50	2							0.75	1	1.50	2		
28.0	133	37.59	50	3.75	5	3.00	4	2.25	3					0.75	1	1.50	2				
14.0	135	16.29	22	1.48	2	2.22	3	0.74	1	0.74	1	0.74	1								
7.0	135	22.96	31	1.48	2	0.74	1	0.74	1							1.48	2				
0.0	170	22.94	39	2.35	4	1.17	2	0.58	1			0.58	1			0.58	1	0.58	1		
pierced	141	31.91	45	2.12	3	1.41	2	0.70	1					0.70	1	0.70	1				
untreated	454	12.11	55	0.66	3	0.66	3	0.22	1			0.22	1	0.22	1			0.44	2		

SUMMARY - ALL DOSE LEVELS

708	31.78	225	2.40	17	1.69	12	1.27	9	0.14	1	0.14	1	0.14	1	0.85	6	0.28	2		

Table 5

Calcium Silicate

in Glycerine: Ethanol

Chi-Square Analyses of Mortality

Dose Level mg/kg	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
7.0	2.18	13.96*(less)	1.55	0.02
14.0	0.33	15.53*(less)	1.20	1.68
28.0	0.01	2.97	8.70*	7.03*
56.0	1.13	12.92*	1.82	14.68*
70.0	0.65	5.21*	5.73*	2.74
100.0	0.25	5.17*	3.23	12.05*
All Doses (DF)	4.19 (6)	102.07*(6)	12.55 (6)	45.49*(6)

* Probability < 0.05-0.005.

Table 6

Calcium Silicate

in Glycerine: Ethanol

Probit Analyses - Mortality

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

Table 7

Calcium Silicate

in Glycerine: Ethanol

Chi-Square Analyses of Abnormalities

Dose Level mg/kg	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
7.0	0.83	0.11	0.62	0.02
14.0	2.38	2.31	3.91*(less)	0.02
28.0	0.11	2.36	0.86	0.14
56.0	0.94	0.14	0.05	0.14
70.0	0.22	0.02	0.02	0.10
100.0	0.25	0.34	0.02	0.09
All Doses (DF)	7.99 (6)	11.87 (6)	7.55 (6)	4.04 (6)

* Probability $<$ 0.05-0.005.

Table 8

Calcium Silicate

in Glycerine: Ethanol

Probit Analyses - Abnormalities

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

Table 9

Calcium Silicate

in Glycerine: Ethanol

Chi-Square Analyses of HLSV Abnormalities

Dose Level mg/kg	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
7.0	0.06	0.18	0.62	0.04
14.0	0.29	1.63	3.91*(less)	0.08
28.0	0.46	3.39	1.94	0.08
56.0	0.31	0.41	0.25	0.07
70.0	0.04	0.07	0.04	0.07
100.0	0.06	0.21	0.02	0.06
All Doses (DF)	9.15 (6)	13.39 (6)	7.87 (6)	1.79 (6)

* Probability < 0.05-0.005.

TABLE 10
CALCIUM SILICATE in GLYCERINE: ETHANOL

TERATOGENIC FINDINGS

TREATMENT	TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
Untreated Control	454	3	1	exencephaly; agenesis-wing, unilateral; cranio-rachischisis-abdomen
			2	hypopigmentation-down
Drilled Control - 0 hrs	150	0	0	
Drilled Control - 96 hrs	202	2	1	celosomia-abdomen
			1	hypopigmentation-down
Pierced Control - 0 hrs	60	2	1	umbilical cord around fetus
			1	dwarfism
Pierced Control - 96 hrs	141	3	1	exencephaly
			1	agenesis-down
			1	malrotation-hindlimb, unilateral
Air Cell - 0 hrs 100.0 mg/kg	38	0	0	
70.0	133	2	1	anophthalmia-bilateral; dysgnathia-beak
			1	hypopigmentation-down
56.0	134	1	1	dwarfism
28.0	135	4	2	celosomia-abdomen
			1	agenisis-head; wing, bilateral; and hindlimb, bilateral
			1	anophthalmia-bilateral; abnormal shortening-maxilla

TABLE 10
CALCIUM SILICATE in GLYCERINE: ETHANOL

TERATOGENIC FINDINGS

TREATMENT	TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
Air Cell - 0 hrs 14.0 mg/kg	131	0	0	
7.0	127	1	1	malrotation-hindlimb, bilateral
0.0	167	5	1	microphthalmia-bilateral; abnormal shortening-maxilla
			2	hypopigmentation-down
			1	umbilical cord around fetus
			1	exencephaly
Air Cell - 96 hrs 100.0 mg/kg	38	0	0	
70.0	176	7	1	hypopigmentation-down
			1	abnormal curvature-toe, unilateral
			1	anophthalmia-bilateral; exencephaly; dysgnathia-beak
			1	dwarfism
			1	fusion failure-abdomen
			1	malrotation-hindlimb, unilateral
			1	abnormal curvature-toe, bilateral
56.0	180	8	1	abnormal curvature-toe, bilateral; dwarfism
			2	abnormal curvature-toe, bilateral
			2	abnormal curvature-toe, unilateral
			1	malrotation-hindlimb, unilateral
			1	buphthalmia-unilateral
			1	micromelia-wing, unilateral

TABLE 10
CALCIUM SILICATE in GLYCERINE: ETHANOL

TERATOGENIC FINDINGS

TREATMENT		TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
Air Cell - 96 hrs	28.0 mg/kg	182	1	1	agenesis-down
	14.0	180	1	1	agenesis-hindlimb, unilateral; abnormal curvature-toe, unilateral; celosomia-abdomen
	7.0	185	4	1	agenesis-head; micromelia-wing, bilateral; dwarfism; celosomia-abdomen
				1	dwarfism
				1	anophthalmia-bilateral;exencephaly; abnormal shortening-maxilla
				1	dwarfism; celosomia-abdomen
	0.0	218	7	1	celosomia-abdomen
				1	exencephaly
				1	exencephaly; abnormal curvature-hindlimb, bilateral and toe, bilateral; celosomia-abdomen
				1	agenesis-down
				1	phocomelia-wing, unilateral and hindlimb, unilateral
				1	phocomelia-hindlimb,unilateral; abnormal curvature-hindlimb, unilateral
				1	anophthalmia-bilateral
Yolk - 0 hrs	100.0 mg/kg	38	1	1	anophthalmia-unilateral; dysgnathia-beak
	70.0	145	5	3	celosomia-abdomen
				1	buphthalmia-bilateral; abnormal shortening-maxilla; dysgnathia-beak; fusion failure-abdomen
				1	umbilical cord around fetus

TABLE 10
CALCIUM SILICATE in GLYCERINE: ETHANOL

TERATOGENIC FINDINGS

TREATMENT	TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
Yolk - 0 hrs 56.0 mg/kg	139	5	2	celosomia-abdomen
		1	1	dwarfism
			1	fusion failure-abdomen
			1	hypopigmentation-down
28.0	140	2	1	hemorrhage
			1	celosomia-abdomen
14.0	146	0	0	
7.0	127	2	1	dwarfism; celosomia-abdomen
			1	fusion failure-abdomen
0.0	187	7	1	agenesis-head
			2	fusion failure-abdomen
			2	abnormal curvature-toe, unilateral
			1	celosomia-abdomen
			1	exencephaly; celosomia-abdomen

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CALCIUM SILICATE in GLYCERINE: ETHANOL
TERATOGENIC FINDINGS

TREATMENT	TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
Yolk - 96 hrs 100.0 mg/kg	38	0	0	
70.0	134	3	2	exencephaly
			1	agenesis-down
56.0	133	5	1	hypopigmentation-down
			1	dysgnathia-beak
			1	hemorrhage
			1	dwarfism
			1	exencephaly
28.0	133	5	1	exencephaly
			1	dwarfism
			1	agenesis-down
			1	anophthalmia-unilateral; dysgnathia-beak
			1	buphthalmia-bilateral; dysgnathia-beak; phocomelia-wing, bilateral
14.0	135	2	1	agenesis-eyelid, unilateral
			1	abnormal curvature-vertebral column; fusion failure-abdomen
7.0	135	2	1	agenesis-down
			1	dysgnathia-beak; dwarfism
0.0	170	4	1	hypopigmentation-down
			1	agenesis-down
			1	fusion failure-abdomen
			1	exencephaly; abnormal shortening-maxilla; dysgnathia-beak