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VITAMIN E
(dl-alpha tocopheryl acetate)

TOXICITY and TERATOGENICITY STUDIES
in Avian Embryos

FDA Contract #72-345

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STUDIES on the TOXICITY and TERATOGENICITY of VITAMIN E

SUMMARY and CONCLUSIONS

Vitamin E was found to be toxic when administered in the air cell prior to incubation at dose levels of 48 mg/kg and above.

Teratological evaluations of vitamin E in the four test protocols failed to demonstrate that this material produced a significant increase in the occurrence of abnormalities at the dose levels employed.

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 25 - 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.

Vitamin E (dl-alpha tocopheryl acetate, 71-58) was dissolved in absolute alcohol for use in the test protocols. The maximum dose level employed was 120 mg/kg (6 mg/egg).

MORTALITY

Mortality data obtained in the four test protocols are shown in Tables 1 - 4. Chi-square analyses of these data indicated a significant increase in embryo deaths only for the air cell-0 hr series (Table 5).

Dose levels of 48 mg/kg and above produced significant increases in mortality in comparison with the absolute alcohol control eggs. Probit analyses of these data did not provide a significant relationship between log dose and probit of mortality (Table 6).

TERATOLOGY

The occurrence of abnormal embryos and those showing H-S-V-L abnormalities in each of the test protocols is shown in Tables 1 - 4. Statistical evaluations of these data failed to indicate that vitamin E was teratogenic in any of the four test protocols at any of the dose levels employed (Tables 7 - 9). The specific teratological findings are listed in Table 10.

TABLE 1

VITAMIN E
In ALCOHOL, DEHYDRATED
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 #
120.0	135	25.92	35	0.74	1	1.48	2	0.74	1			0.74	1								
96.0	99	25.25	25	1.01	1	1.01	1							1.01	1						
48.0	99	23.23	23	1.01	1	1.01	1					1.01	1								
32.0	96	15.62	15	2.08	2	1.04	1							1.04	1	1.04	1				
16.0	100	10.00	10	1.00	1	1.00	1					1.00	1								
0.0	134	11.94	16	0.74	1	0.74	1					0.74	1								
drilled	100	7.00	7	0.00	0	0.00	0														
untreated	397	11.58	46	0.00	0	0.00	0														

SUMMARY - ALL DOSE LEVELS

529	20.42	108	1.13	6	1.13	6	0.19	1			0.57	3	0.38	2	0.19	1				

TABLE 2

VITAMIN E
in ALCOHOL, DEHYDRATED
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 #
120.0	108	48.14	52	3.70	4	1.85	2					1.85	2			0.92	1			0.92	1
96.0	108	45.37	49	0.92	1	0.00	0													0.92	1
48.0	107	45.79	49	1.86	2	1.86	2	0.93	1					0.93	1						
32.0	106	48.11	51	0.94	1	0.94	1					0.94	1								
16.0	109	49.54	54	1.83	2	1.83	2	0.91	1			0.91	1								
0.0	106	45.28	48	2.83	3	3.77	4	0.94	1			1.88	2	0.94	1						
drilled	182	8.79	16	0.54	1	0.00	0									0.54	1				
untreated	397	11.58	46	0.00	0	0.00	0														

SUMMARY - ALL DOSE LEVELS

538	47.40 255	1.86 10	1.30 7	0.37 2		0.74 4	0.19 1	0.19 1		0.37 2	

TABLE 3

VITAMIN E
in ALCOHOL, DEHYDRATED
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 #
120.0	92	53.26	49	3.26	3	3.26	3	2.17	2			1.08	1			1.08	1				
96.0	135	45.92	62	0.74	1	0.00	0											0.74	1		
48.0	94	42.55	40	1.06	1	1.06	1					1.06	1			1.06	1				
32.0	97	38.14	37	0.00	0	0.00	0														
16.0	97	40.20	39	1.03	1	1.03	1	1.03	1												
0.0	57	40.35	23	0.00	0	0.00	0														
pierced																					
untreated	397	11.58	46	0.00	0	0.00	0														

SUMMARY - ALL DOSE LEVELS

515	44.08 227	1.17	6	0.97	5	0.58	3		0.39	2		0.39	2	0.19	1	

TABLE 4

VITAMIN E
in ALCOHOL, DEHYDRATED
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 #
120.0	222	37.38	83	2.70	6	1.35	3	0.90	2			0.45	1			0.90	2			0.45	1
96.0	148	23.64	35	0.00	0	0.00	0														
48.0	150	20.66	31	0.66	1	0.00	0											0.66	1		
32.0	149	27.51	41	0.67	1	0.67	1	0.67	1												
16.0	146	23.97	35	0.68	1	0.00	0									0.68	1				
0.0	171	28.07	48	1.75	3	2.33	4	1.75	3					0.58	1						
pierced	160	20.00	32	1.25	2	1.25	2	0.62	1			0.62	1								
untreated	397	11.58	46	0.00	0	0.00	0														

SUMMARY - ALL DOSE LEVELS

815	27.61	225	1.10	9	0.49	4	0.37	3			0.12	1			0.37	3	0.12	1	0.12	1

TABLE 5

VITAMIN E
In ALCOHOL, DEHYDRATED
CHI-SQUARE ANALYSES of MORTALITY

Dose Level mg/kg	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
16.0	0.07	0.24	0.02	0.49
32.0	0.37	0.08	0.01	0.00
48.0	4.43*	0.00	0.01	1.98
96.0	6.07*	0.02	0.30	0.59
120.0	7.68*	0.08	1.86	3.37
All Doses (DF)	18.37*(5)	0.70(5)	5.70(5)	16.35*(5)

* Probability $<$ 0.05 - 0.005.

TABLE 6

VITAMIN E
in ALCOHOL, DEHYDRATED
PROBIT ANALYSES - MORTALITY

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

TABLE 7

VITAMIN E
in ALCOHOL, DEHYDRATED
CHI-SQUARE ANALYSES of ABNORMALITIES

Dose Level mg/kg	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
16.0	0.26	0.00	0.07	0.12
32.0	0.09	0.26	0.00	0.13
48.0	0.25	0.00	0.06	0.14
96.0	0.25	0.27	0.20	1.08
120.0	0.50	0.00	0.60	0.08
All Doses (DF)	1.23(5)	3.21(5)	6.09(5)	7.40(5)

TABLE 8

VITAMIN E
in ALCOHOL, DEHYDRATED
PROBIT ANALYSES - ABNORMALITIES

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

TABLE 9

VITAMIN E
In ALCOHOL, DEHYDRATED
CHI-SQUARE ANALYSES of HLSV ABNORMALITIES

Dose Level mg/kg	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
16.0	0.26	0.00	0.07	1.05
32.0	0.23	0.26	0.00	0.13
48.0	0.25	0.00	0.06	1.10
96.0	0.25	1.39	0.00	1.08
120.0	0.50	0.00	0.60	0.01
All Doses (DF)	0.13(5)	3.28(5)	8.66(5)	7.12(5)

TABLE 10

VITAMIN E in ALCOHOL, DEHYDRATED
TERATOGENIC FINDINGS

TREATMENT		TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
Untreated Control		397	0	0	
Drilled Control - 0 hrs		100	0	0	
Drilled Control - 96 hrs		182	1	1	agenesis-down
Pierced Control - 96 hrs		160	2	1	fusion failure-abdomen
				1	dysgnathia-beak
Air Cell - 0 hrs	120 mg/kg	135	1	1	anophthalmia; exencephaly; abnormal shortening-maxilla; celosomia-abdomen
	96.0	99	1	1	agenesis-hindlimb, bilateral
	48.0	99	1	1	celosomia-abdomen
	32.0	96	2	1	abnormal curvature-toe
				1	dwarfism
	16.0	100	1	1	celosomia-abdomen
	0.0	134	1	1	celosomia-abdomen
Air Cell - 96 hrs	120.0	108	4	1	fusion failure-abdomen
				1	cachexia
				1	celosomia-abdomen
				1	agenesis-down
	96.0	108	1	1	cachexia

TABLE 10

VITAMIN E in ALCOHOL,DEHYDRATED

TERATOGENIC FINDINGS

TREATMENT		TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
Air Cell - 96 hrs	48.0 mg/kg	107	2	1	abnormal curvature-toe, unilateral
				1	dysgnathia-beak
	32.0	106	1	1	celosomia-abdomen
	16.0	109	2	1	fusion failure-abdomen
				1	anophthalmia-unilateral; dysgnathia-beak
	0.0	106	3	1	anophthalmia-unilateral; dysgnathia-beak; celosomia-abdomen
				1	celosomia-abdomen
				1	malrotation-hindlimb, unilateral
Yolk - 0 hrs	120.0	92	3	1	anophthalmia-bilateral; microcephaly; dysgnathia-beak; dwarfism
				1	celosomia-abdomen
				1	exencephaly
	96.0	135	1	1	umbilical cord around fetus
	48.0	94	1	1	dwarfism; celosomia-abdomen
	32.0	97	0	0	
	16.0	97	1	1	anophthalmia-bilateral; abnormal shortening-maxilla
	0.0	57	0	0	

Sheet 3

VITAMIN E in ALCOHOL, DEHYDRATED
TERATOGENIC FINDINGS

TREATMENT		TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
Yolk - 96 hrs	120.0 mg/kg	222	6	2	anophthalmia-unilateral; dysgnathia-beak
				1	celosomia-abdomen
				1	dwarfism
				1	agenesis-down
				1	cachexia
	96.0	148	0	0	
	48.0	150	1	1	hemorrhage-umbilical cord
	32.0	149	1	1	exencephaly
	16.0	146	1	1	dwarfism
	0.0	171	3	1	buphthalmia-unilateral
				1	exencephaly
				1	buphthalmia-unilateral; dysgnathia-beak; abnormal shortening-mandible; phocomelia-wing, unilateral; agenesis-hindlimb, unilateral