

Investigation of the Toxic & Teratogenic Effects of GRAS Substances to the Developing  
Chicken Embryo-Report of the investigation of Sorbitol in the Developing Chicken  
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SUBJECT: Investigation of the Toxic and Teratogenic Effects of  
GRAS Substances to the Developing Chicken Embryo

Attached is the report of the investigation of SORBITOL  
in the developing chicken embryo.

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Investigations of the Toxic and Teratogenic Effects of  
GRAS Substances to the Developing Chicken Embryo:

SORBITOL

PROTOCOL:

Sorbitol (1) was tested for toxic and teratogenic effects to the developing chicken embryo under four sets of conditions. It was administered with water as the solvent by two routes and at two stages of embryonic development; via the air cell at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and at 96 hours using techniques that have been described previously (2, 3).

Groups of ten or more eggs were treated under these four conditions at several dose levels until a suitable total number of eggs per level was reached for all levels allowing some to hatch. Groups of adequate size were treated solely with the solvent at corresponding volumes. Untreated controls were also included in each experiment.

After treatment, all the eggs were candled daily and the non-viable embryos were removed. Surviving embryos were allowed to hatch. Hatched chicks and non-viable embryos were examined grossly for abnormalities (internally and externally) as well as for toxic responses such as edema and hemorrhage. Along with these, histological examinations of major organs (liver, heart, kidney, lung, brain, intestine, gonad, and some endocrine organs) were carried out by taking samples from a representative number of animals from each experimental group.

RESULTS:

The results obtained are presented in Tables 1 through 4 for each of the four conditions of the test.

Columns 1 and 2 give the dose administered in milligrams per egg and milligrams per kilogram egg weight, respectively. (The milligrams per kilogram figure is based on an average egg weight of fifty grams.)

Column 3 is the total number of eggs treated.

Column 4 is the percent mortality, i. e., the total number of non-viable eggs divided by the total number of treated eggs.

Column 5 is the total number of abnormal birds expressed as a percentage of the total number of eggs treated. This includes all the abnormalities observed and also the toxic responses such as edema,

hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia, and neural disorders including ataxia.

Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton expressed as a percentage of the total number of eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

Columns 3 through 6 have been corrected for accidental deaths if any occurred. Included in these columns are comparable data for the solvent-treated eggs and the untreated controls.

The mortality data in column 4 has been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (4). The results obtained are indicated at the bottom of each table.

The data in columns 4, 5 and 6 has been analyzed using the Chi Square test for significant differences from the solvent background. Each dose level is compared to the solvent value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

#### DISCUSSION:

Sorbitol was found not to be embryotoxic when administered to the embryo at the pre-incubation stage with the dose level at or below 25 mg/egg. The toxicity, however, was significantly ( $P=0.05$ ) greater than solvent-treated eggs at a higher dose level, namely, 40 mg/egg. On the other hand, sorbitol was toxic at all dose levels when administered at 96 hours of incubation except at 1.0 mg/egg and 10.0 mg/egg via the yolk. Probit analysis resulted in an  $LC_{50}$  of 103.71 mg/egg (yolk at 0 hours, Table 3). The air cell treatment at both stages and the yolk treatment at 96 hours each resulted in a line whose slope was not significantly different from zero (Tables 1, 2 and 4).

Abnormal birds were seen under all of the conditions of the test, although the incidence of birds having a structural abnormality of the head, limbs, viscera, or skeleton was not significantly different from that of the solvent background ( $P=0.05$ ). Of the 60 control eggs four abnormal birds were produced, all with curled toes.

**AIR CELL AT 0 HOURS:** Abnormalities were found in all the dose levels. At 40.0 mg/egg, five abnormal birds, all with curled toes, were found. At 25.0 mg/egg, seven birds with abnormalities were found; four had curled toes and three had celosomia. At 10.0 mg/egg, four abnormal birds, three with curled toes and one with hypopigmented down, were seen. At 5.0 mg/egg, five birds, two with curled toes and three with hip contractures,

were found. At 1.0 mg/egg, three birds had hip contractures and four had curled toes. The solvent-treated birds showed five abnormal birds, four with curled toes and one with hip contracture.

**AIR CELL AT 96 HOURS:** All of the test levels produced abnormalities. The solvent-treated had two abnormal birds with curled toes and one abnormal bird with hip contractures. At 40.0 mg/egg, three birds had hip contractures and two had curled toes. At 25.0 mg/egg, four abnormal birds were found; three with curled toes and one with hip contractures. At 10.0 mg/egg, one bird showed hip contracture and four exhibited curled toes. At 5.0 mg/egg, five abnormal birds were found; three with hip contractures and two with curled toes. At the 1.0 mg/egg level four birds, three with curled toes and one with hip contracture, were seen.

**YOLK SAC AT 0 HOURS:** All dose levels tested showed abnormalities. At 40.0 mg/egg four abnormal birds were produced; two had curled toes and two had hip contractures. At 25.0 mg/egg, four birds with curled toes and one bird with hip contractures were found. At 10.0 mg/egg, the abnormal birds consisted of two birds with hip contracture and one with curled toes. At 5.0 mg/egg four abnormalities were found, three birds with curled toes and one with hip contracture. At 1.0 mg/egg only one abnormal bird was found. It had curled toes. The solvent-treated birds produced five birds with curled toes and two birds with hip contractures.

**YOLK SAC AT 96 HOURS:** Abnormalities were found at all tested dosages. At 40.0 mg/egg only one abnormality was found. It was a bird with curled toes. At 25.0 mg/egg four birds with hip contractures and one hypopigmentation of the down were produced. At 10.0 mg/egg three abnormal birds were found. All had curled toes. At 5.0 mg/egg the three abnormal birds that were produced had curled toes. At 1.0 mg/egg only one abnormal bird was found. It had curled toes. In the solvent-treated eggs five abnormal birds, all with curled toes, were found.

Microscopical examination of the paraffin embedded and H&E stained sections revealed no consistent histological changes in any of the organs observed. Although occasional hemorrhage, vacuolization, or fatty infiltration in the liver were seen, none of these changes correlated with the administered dose nor the varieties of the external abnormalities.

Judging from all of these test results, it is concluded that sorbitol is not teratogenic to the chicken embryo even at relatively high dosages. Most of the abnormalities found in this test were non-specific, i. e., they were found also in the solvent-treated or untreated controls.

1. Sorbitol, U.S.P., crystalline, powdered, FDA 71-31, Atlas Chemical Works, St. Louis, Lot #ZPA
2. McLaughlin, J., Jr., Marliac, J.-P., Verrett, M.J., Mutchler, M.K. and Fitzhugh, O.G. Toxicol. Appl. Pharmacol. 5:760-770, 1963
3. Verrett, M.J., Marliac, J.-P. and McLaughlin, J., Jr. JAOAC 47: 1002-1006, 1964
4. Finney, D.J. Probit Analysis, 2nd ed., Cambridge Press, Cambridge, Appendix I, 1964

Table 1  
Sorbitol  
Air Cell at 0 Hours

Dose		Number of eggs	Percent Mortality	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
40.0	800	76	50.00*	10.52	6.57
25.0	500	74	33.78	13.51	9.45
10.0	200	74	35.13	5.40	4.05
5.0	100	75	40.00	6.66	6.66
1.0	20	72	22.22	9.72	9.72
Water		50	24.00	10.00	10.00
Control		60	18.33	6.66	6.66

P (calculated) < P (0.05)

\*Significantly different from solvent  $P \leq 0.05$

Table 2

Sorbitol

Air Cell at 96 Hours

Dose		Number of eggs	Percent Mortality	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
40.0	800	72	45.83*	6.94	6.94
25.0	500	77	40.25*	7.79	5.19
10.0	200	73	45.20*	6.84	6.84
5.0	100	74	35.13*	9.45	6.75
1.0	20	71	40.84*	5.63	5.63
Water		48	14.58	6.25	6.25
Control		60	18.33	6.66	6.66

P (calculated) < P (0.05)

\*Significantly different from solvent  $P \leq 0.05$

Table 3

## Sorbitol

## Yolk at 0 Hours

Dose		Number of eggs	Percent Mortality	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
40.0	800	71	57.74*	5.63	5.63
25.0	500	76	46.05	7.89	6.57
10.0	200	76	42.10	5.26	3.94
5.0	100	75	36.00	8.00	5.33
1.0	20	74	35.13	1.35	1.35
Water		50	28.00	12.00	12.00
Control		60	18.33	6.66	6.66

LC<sub>30</sub> 26.430 mg/egg (528.605 mg/kg)

LC<sub>50</sub> 103.717 mg/egg (2074.342 mg/kg)

LC<sub>90</sub> 2930.443 mg/egg (58608.878 mg/kg)

\*Significantly different from solvent  $P \leq 0.05$

Table 4

Sorbitol

Yolk at 96 Hours

Dose		Number of eggs	Percent Mortality	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
40.0	800	70	57.14*	2.85	1.42
25.0	500	68	48.52*	7.35	5.88
10.0	200	69	37.68	7.24	4.34
5.0	100	69	44.92*	4.34	4.34
1.0	20	75	41.33	2.66	1.33
Water		55	25.45	9.09	9.09
Control		60	18.33	6.66	6.66

P (calculated) < P (0.05)

\*Significantly different from solvent P ≤ 0.05