Investigations of the Toxic & Teratogenic Effects of GRAS Substances on the Developing Chick Embryo (Stannous Chloride) No Date

C28

Tab 4

Investigations on the Toxic and
Teratogenic Effects of GRAS
Substances on the Developing Chick Embryo.[1]

Stannous Chloride

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[1] Report of investigations conducted under Contract No. 72-343 with the Food and Drug Administration, PHS, DHEW.

General Protocol:

Ten test substances were supplied by the Food and Drug Administration for testing in the chick embryo. Details on the nature and source of these substances is shown in Table 1. All substances were stored at room temperature in the dark until they were used, except that the propyl gallate and phosphated mono- and di-glycerides were kept under refrigeration. Most of the substances were dissolved in a suitable solvent or suspended in a suitable liquid for injection into fertile eggs. In one instance the substance was injected directly without a solvent or carrier. Specific information about solvents, [illegible] of the substances and problems peculiar to individual substances will be given under specific protocol for each substance tested.

Fertile eggs used in these investigations were from a specific pathogen free flock of Dekalb 161 egg production type chickens fed a breeder ration free of antibiotics or other drugs. Eggs were stored at 55° F and a relative humidity of 80 percent for 0 to 5 days prior to use. Eggs were allowed to reach room temperature, placed on plastic flats and subjected to ultraviolet irradiation for 30 minutes. The top of each egg was cleansed by a cotton swab saturated with 70 percent ethanol, a small hole was drilled over the air cell through the shell and the test substance was injected with the aid of a 0.25 ml. tuberculin syringe fitted with a suitable needle. All equipment and glassware used to handle the test substances or their solutions or suspensions were sterilized by auto claving and every attempt was made to avoid microbiological contamination of the eggs. Following injection the hole in each egg was sealed by a drop of flexible collodion and the eggs were set in or returned to the incubators. Jamesway [illegible] 252 Incubator-Hatchers were used and maintained at 100° F dry bulb temperature and 86° F wet bulb temperature during the first 18 days of incubation. Eggs were turned automatically each 4 hours. Eggs were candled periodically to remove dead embryos and all embryos were examined for stage of development and obvious defects. After 18 days of incubation viable embryos were transferred to hatching baskets and hatching temperature was reduced [illegible] increased to a 90° F wet bulb [illegible]. [illegible] (1 [illegible] day) chicks were examined for abnormalities and [illegible] were cleared and [illegible] stained to examine them for skeletal defects. Other embryos (50 for each substance studied) were sacrificed and samples of liver, [illegible], [illegible], brain, eye, spleen, heart, pancreas, lung and kidney were taken and fixed in formalin. Later tissues were embeded in paraffin, cut, stained and mounted for histopathological examination. Each sample was done in [illegible] and hence a total of 10,000 tissues were examined for lesions.

Preliminary range finding experiments were conducted to find the doses of the test substances that could be used in constructing dose response curves for toxicity as measured by embryonic mortality. In two cases, the test substance was non-toxic in the largest dose that could be accommodated by injection. Specific dose response experiments using 100 or more eggs per dose and 5 or more doses of the test substance were conducted at a minimum of 3 time intervals to obtain the toxicity data reported. Solvent or sham injected controls and untreated control groups of eggs were used with each experiment. In some cases, extra trials were conducted to provide embryos for examination at critical [illegible] of the test substances in order to further evaluate teratogenic [illegible] additional data on the nature of embryonic defects.

Data obtained from the experiments (except that from the range finding [illegible]) was transferred to data sheets provided (PHS form 2572, 2572a and [illegible]) and utilized to FDA for statistical analysis. Nine types of data [illegible] summarizing 2 statistical treatments of the data were provided by FDA [illegible]. The results presented and interpretations made are largely based on these data summaries.

Table 1

FDA Project Test Substances

Test Substance and Identification		Compound No.
1. Lecithin, Edible Dermost Dairies, Inc. Appleton, Wisc.		000063423
2. Propyl Gallate Lot 337		000121799
3. Sodium Ascorbate, U.S.P. FCC Lot No. 965102 Hoffmann-LaRoche Inc., Nutley, N. J. FDA 3-67 73(C)		000134032
4. Sodium Erythorbate F.C.C. Lot No. 634072 FDA 3167 73(C) Hoffmann-LaRoche, Nutley, N. J.		977052064
5. Oil Nutmeg NF, East Indian Triessche Dodge & Olcott, Inc. 71-28 New York, N. Y.		MX 8008455
6. Zinc Sulfate - Rayon [illegible] Virginia Chemicals, Inc. Portsmouth, Va.	Anhyd. Monohyd.	007733020 007446197
7. Stannous Chloride, AR 2H2O Mallinckrodt Chemical Works St. Louis, Mo.		007772998
8. Talc USP #141, Whittaker, Clark and Daniels, Inc.		010101390
9. Carob Bean Gum FDA 71-14		PM 9000402
10. Phosphated Mono- and Di-Glycerides Lot No. 126 Witco Chemical Organics Division New York, N. Y. EMCOL M70-30C		977051323

General Discussion and Comparisons:

A comparison of the relative toxicity of the ten compounds tested is shown in Table ii. When toxicity is evaluated by the air cell route of injection at 96 hrs. of incubation, which was the most sensitive for most of the substances tested, it may be seen that the test substances can be divided into 3 categories of toxicity. Substances highly toxic are zinc sulfate, propyl gallate and guar gum. Moderate toxicity was encountered with sodium ascorbate, sodium erythorbate, oil of nutmeg and stannous chloride. Those substances of low toxicity were lactose, talc and phosphated mono- and di-glyceride.

Most of the substances tested produced general embryo toxic response as ascites and/or edema except for lactose and talc at the doses tested. Some specific structural defects were noted and seemed to be related to certain substances as shown in Table ii.

Table ii

Comparison of Ten Substances Tested
for Toxicity and Teratology

Substance Tested	LC_{50} via air cell at 96 hrs.	Specific Abnormalities Noted
Lactose	very large	none
Propyl Gallate	13 mgs./kg.	Ascites, edema, celosomia.
Sodium Ascorbate	100 mgs./kg.	Ascites, edema, celosomia, liver histopathology, head defects.
Sodium Erythorbate	34 mgs./kg.	Ascites, liver histopathology.
Oil of Nutmeg	240 mgs./kg.	Ascites, edema, celosomia, dwarfism.
Zinc Sulfate	4 mgs./kg.	Ascites, edema, celosomia, dwarfism.
Stannous Chloride	120 mgs./kg.	Ascites, edema, celosomia.
Talc	>200 mgs./kg.	none
Carob Bean Gum	23 mgs./kg.	Anophthalmia, phocomelia, micromelia, torticollis, celosomia.
Phosphated Dipo-[illegible]	[illegible] mgs./kg.	Ascites, [illegible], brachygnathia.

VII. STANNOUS CHLORIDE DIHYDRATE

Specific Protocol:

While stannous chloride is extremely soluble in less than its own weight of water, it forms an insoluble basic salt on dilution to useful test concentrations. Use of hydrochloric acid to prevent formation of the insoluble [illegible] led to high embryonic mortality in the acid injected controls. Hence a highly concentrated solution of stannous chloride was prepared in sterile water and then diluted in propylene glycol to useful test concentrations. Propylene glycol was toxic at 96 hrs. via the air cell but useful data were obtained. Five dose levels of stannous chloride dihydrate were tested both at 0 and 96 hrs. of incubation and via both air cell and yolk routes of administration.

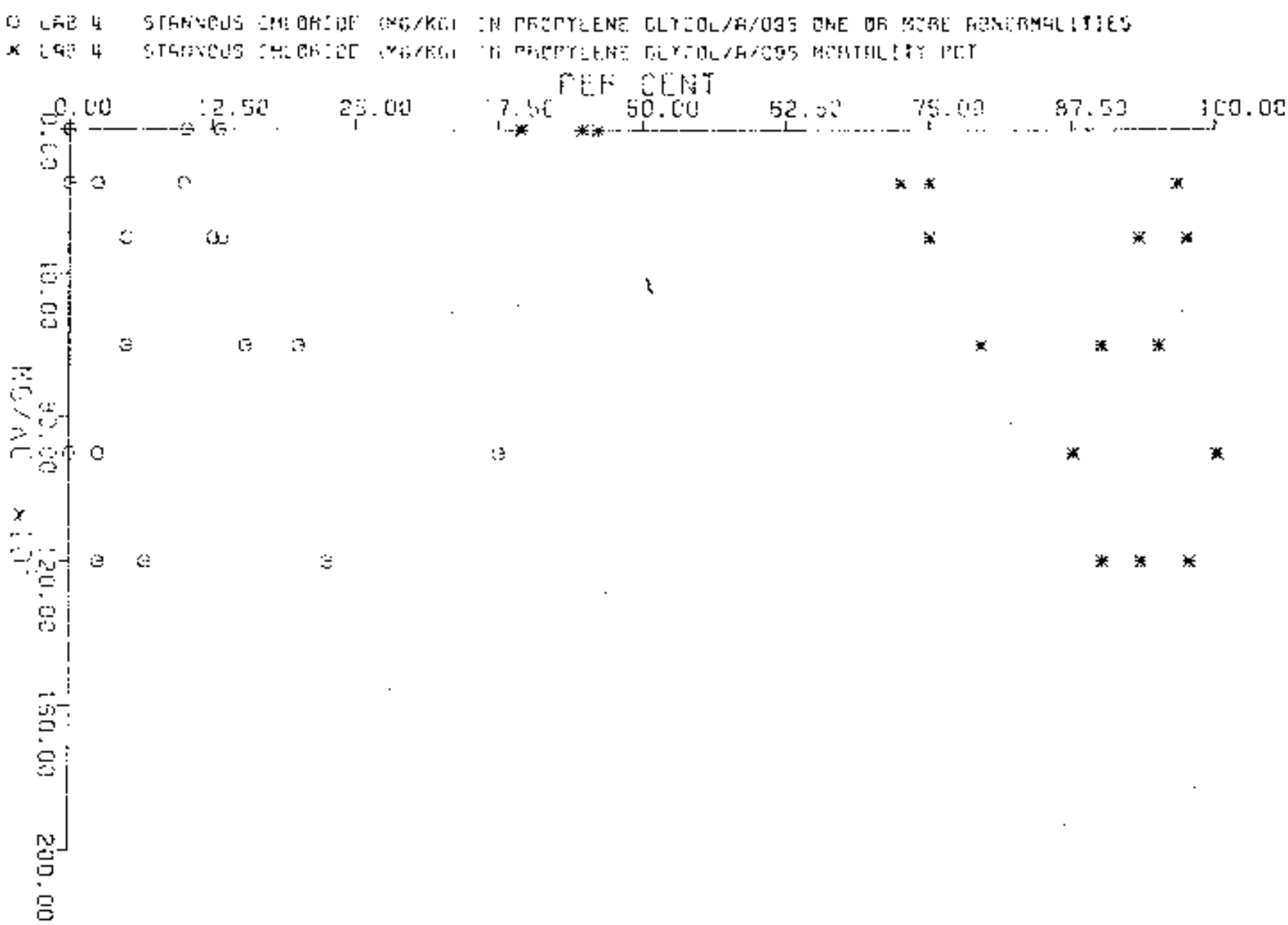
Results:

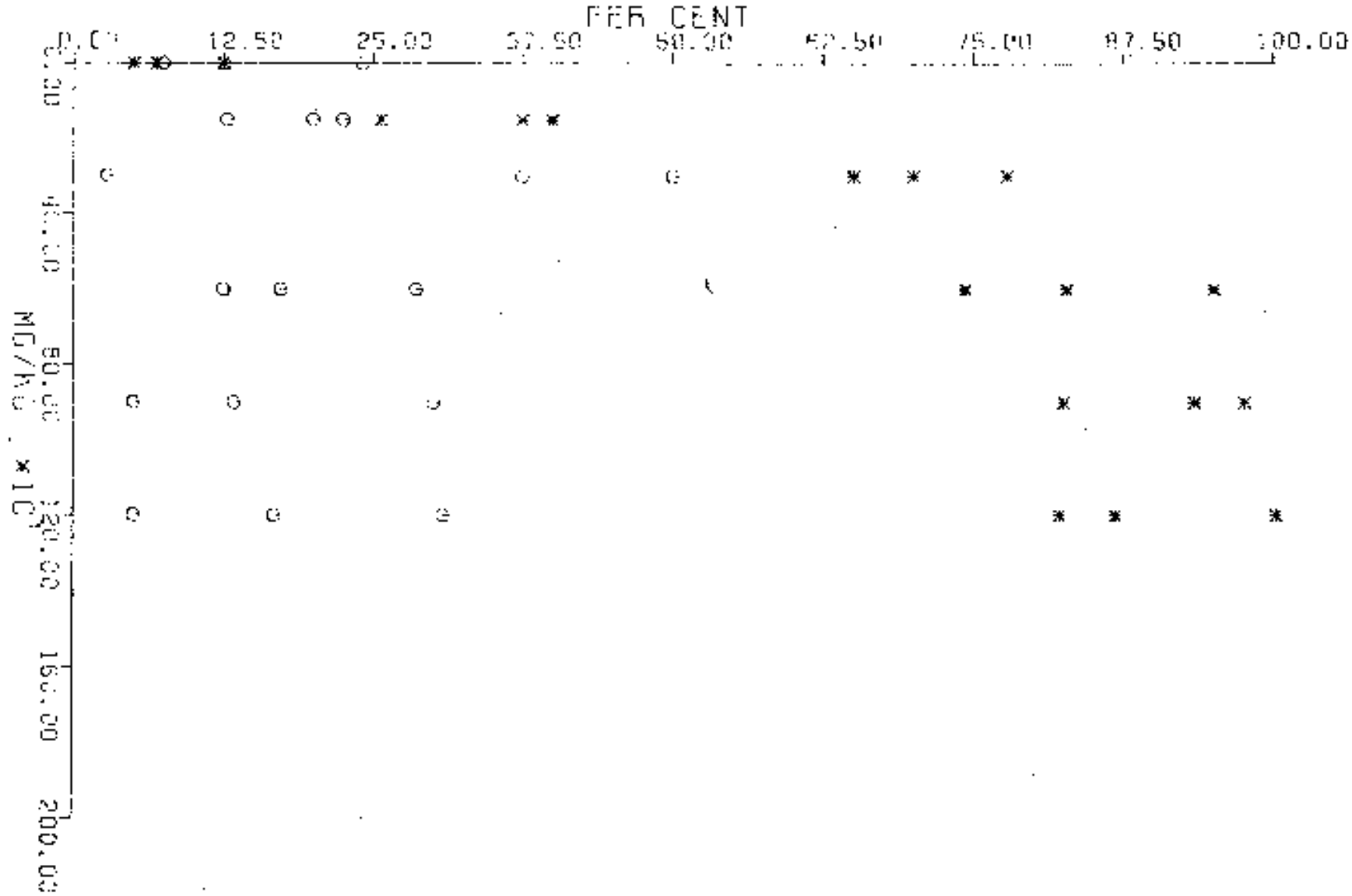
The data for stannous chloride dihydrate is presented in Tables 25-28. Percent mortality due to compound administration was significantly increased at most dose levels. The regression of dose on mortality was highly significant when given at 0 hr. via the air cell and was significant when given at 0 hr. via the yolk. Mortality increased rapidly over the two lowest dose levels and then increased more slowly at higher doses without reaching 100 percent. Percent abnormal chicks hatched was increased significantly by 15 mgs./egg at 0 hr. via the air cell and at 7.5, 30, 45 and 60 mgs./egg at 96 hrs. via the yolk but not by other conditions of administration. Percent H-S-V-L abnormalities was significantly increased by most of the same treatments mentioned above except that the increase was not great enough for significance in the first case but became significant for the 30 mg./egg level when given at 96 hrs. via the air cell. With 96 hrs. yolk treatment the regression of dose [illegible] Ascites, edema and celosomia were more [illegible] in the embryos treated with stannous chloride and again the most [illegible] H-S-V-L defect was celosomia. In the area of histopathology the incidence of muscle atrophy, edema and degeneration was increased in this study but no relationship to treatment was apparent.

Discussion:

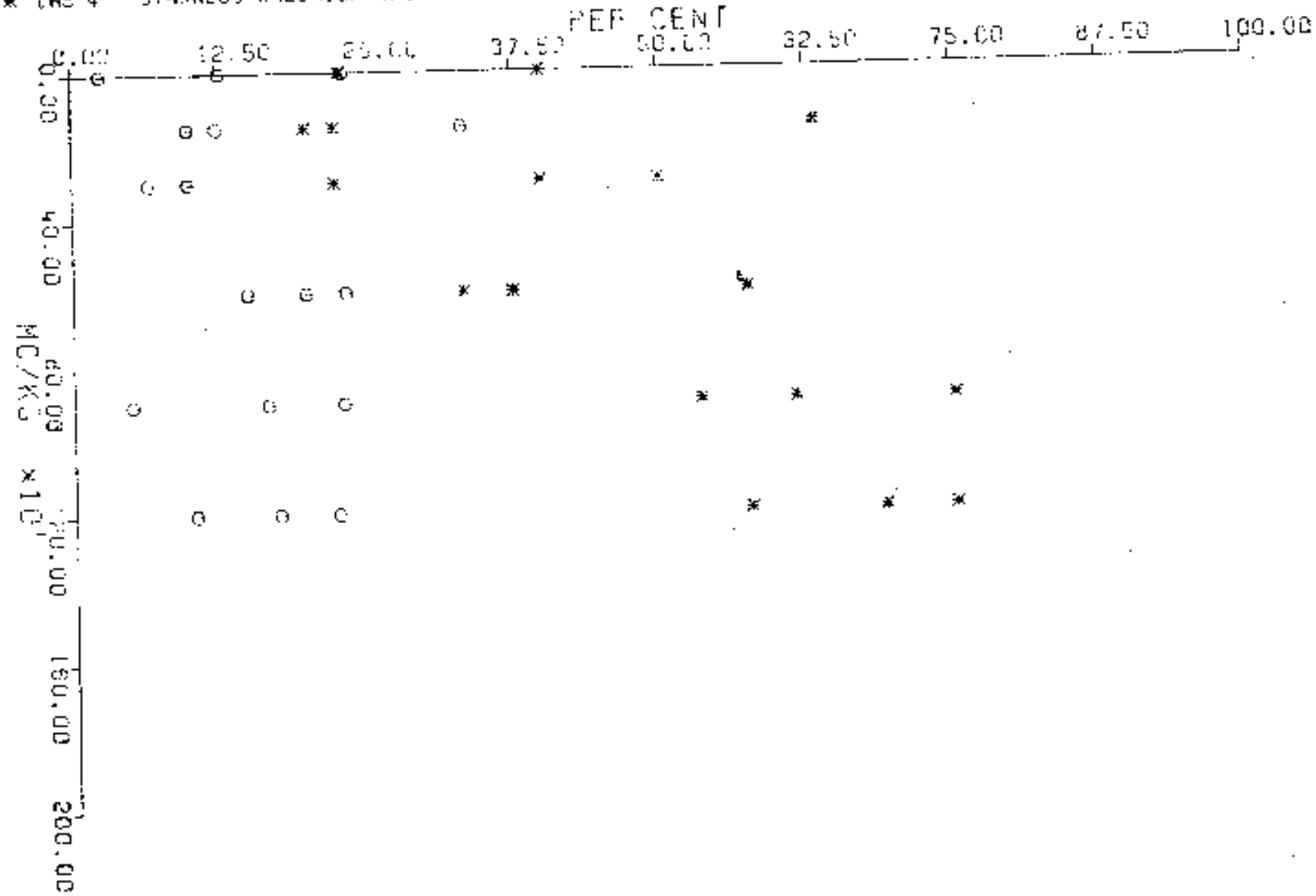
Stannous chloride clearly produces an embryo toxic response at lower levels of administration that is not greatly increased by higher levels of the compound. This seems to be true both for percent mortality and percent abnormal chicks hatched. The LC_{50} at 96 hrs. via the air cell was about 120 mgs./kg. taken from the hatchability curves.

Tab 4


O LAB 4 STANNOUS CHLORIDE (MG/KG) IN PROPYLENE GLYCOL/A/035 ONE OR MORE ABNORMALITIES
* LAB 4 STANNOUS CHLORIDE (MG/KG) IN PROPYLENE GLYCOL/A/035 MORTALITY PCT
PER CENT
0.00
12.50
25.00
37.50
50.00
62.50
75.00
87.50
100.00
MG/AC
0.00
40.00
80.00
120.00
160.00
200.00


O LAB 4 STANNOUS CHLORIDE (MG/KG) IN PROPYLENE GLYCOL/A/200 ONE OR MORE ABNORMALITIES
* LAB 4 STANNOUS CHLORIDE (MG/KG) IN PROPYLENE GLYCOL/A/200 MORTALITY PCT
PER CENT
0.00
12.50
25.00
37.50
50.00
62.50
75.00
87.50
100.00
MG/KG * 10
0.00
40.00
80.00
120.00
160.00
200.00

Lab 4


O LAB 4 STANNOUS CHLORIDE (MG/KG) IN PROPYLENE GLYCOL/Y/200 ONE OR MORE ABNORMALITIES
* LAB 4 STANNOUS CHLORIDE (MG/KG) IN PROPYLENE GLYCOL/Y/200 MORTALITY PCT
PER CENT
0.00
12.50
25.00
37.50
50.00
62.50
75.00
87.50
100.00
MG/KG
0.00
40.00
80.00
120.00
160.00
200.00
X10

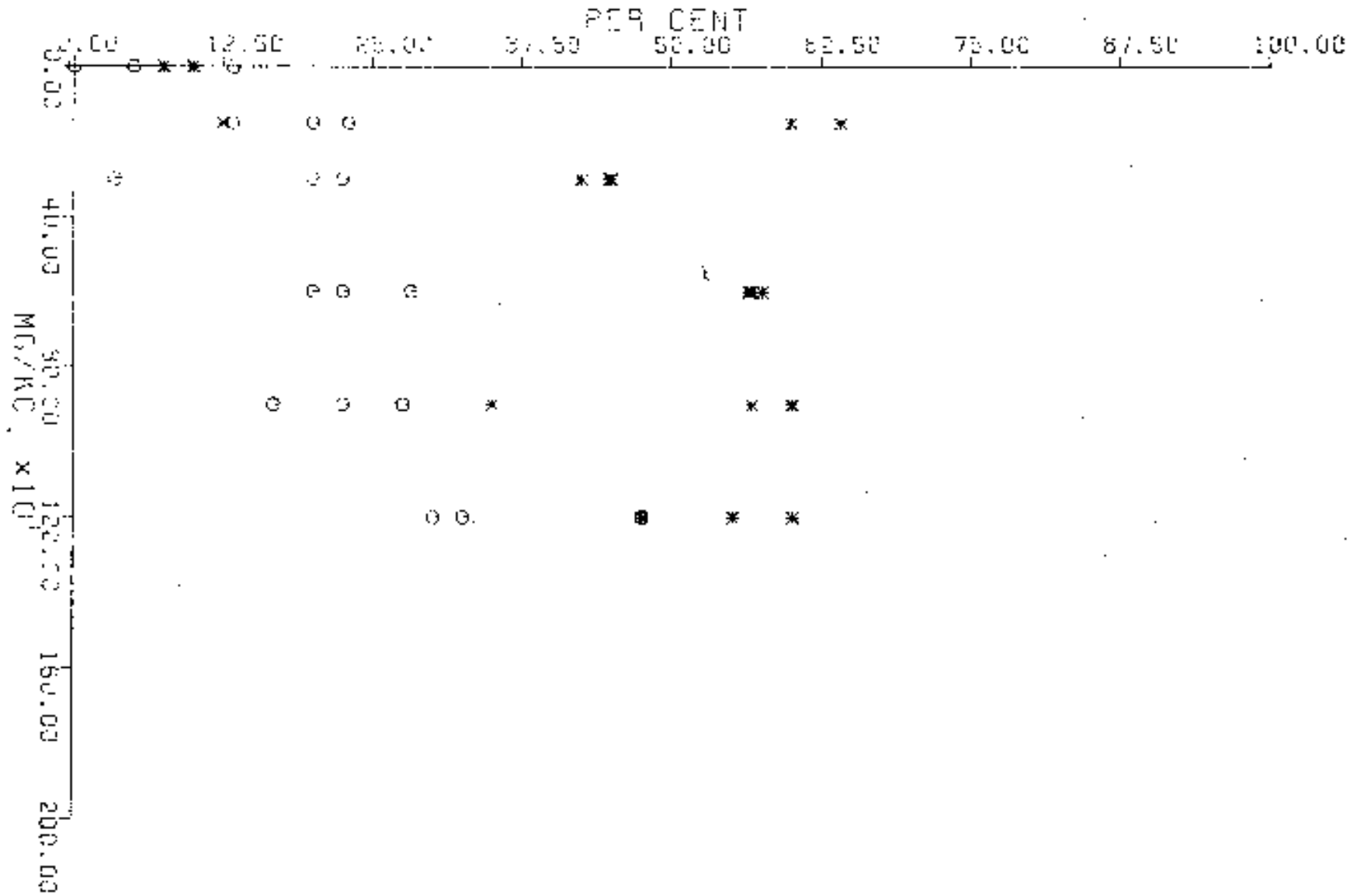

O LAB 4 STANNOUS CHLORIDE (MG/KG) IN PROPYLENE GLYCOL/Y/296 ONE OR MORE ABNORMALITIES
* LAB 4 STANNOUS CHLORIDE (MG/KG) IN PROPYLENE GLYCOL/Y/296 MORTALITY PCT
PER CENT
0.00
12.50
25.00
37.50
50.00
62.50
75.00
87.50
100.00
40.00
80.00
120.00
160.00
200.00
MG/KG, x10

Table 25

DATA SUMMARY

Stannous Chloride in Prop Glycol
via Air Cell at 3 Hr.

Dose of Compound Injected (mgs./kg.)	(mgs./egg)	Number of Eggs	Percent Mortality[4]	Percent Abnormal Chicks Hatched[5]	Percent H-S-V-L Abnormalities
Control	None	438	6.84	6.39	1.59
Solvent	None	109	8.25	13.76	1.83
150.0	7.5	109	33.94[1]	18.34	2.75
300.0	15.0	106	70.75[1]	29.24[2]	7.54[3]
600.0	30.0	104	84.62[1]	19.23	5.76
900.0	45.0	110	90.90[1]	16.36	4.54
1200.0	60.0	100	89.00[1]	17.43	5.50

[1] Difference from control test is highly significant

[2] Difference from control group response is highly significant

[3] [illegible]

[4] Regression of dose on mortality is highly significant

LC_{30} = 127 mg/kg
LC_{50} = 229 mg/kg
LC_{70} = 414 mg/kg
LC_{90} = 971 mg/kg

[5] Slope is negative

Table 26

DATA SUMMARY

Stannous Chloride in Prop Glycol
via Air Cell at 96 Hrs.

Dose of Compound Injected (mgs./kg.)	(mgs./egg)	Number of Eggs	Percent Mortality[4]	Percent Abnormal Chicks Hatched[5]	Percent H-S-V-L Abnormalities
Control	None	438	6.84	6.39	1.59
Solvent	None	106	43.39	11.32	0.94
150.0	7.5	110	80.00[1]	4.54	0.90
300.0	15.0	110	88.18[1]	10.00	3.63
600.0	30.0	109	88.07[1]	17.43[2]	10.09[3]
[illegible]	45.0	110	[illegible].45[1]	14.54	3.63
[illegible]	60.0	110	[illegible].63[1]	10.90	5.45

1 Difference from control test is highly significant

2 [illegible]

3 Difference from control group response is significant

4 [illegible] - F (Cal) < F (.05)

5 Slope is negative

Table 27

DATA SUMMARY

Stannous Chloride in Prop Glycol
via Yolk at 0 Hr.

Dose of Compound Injected (mgs./kg.)	(mgs./egg)	Number of Eggs	Percent Mortality[4]	Percent Abnormal Chicks Hatched[5]	Percent H-S-V-L Abnormalities
Control	None	438	6.84	6.39	1.59
Solvent	None	108	27.77	16.66	0.92
150.0	7.5	110	32.72	17.27	4.54
300.0	15.0	110	37.27	9.09	1.81
600.0	30.0	110	43.63[1a]	19.09	2.72
[illegible]	[illegible]	109	[illegible][1]	19.34	2.75
[illegible]	[illegible]	[illegible]	[illegible][1]	[illegible][2]	4.58[3]

[1] Difference from control test is highly significant

[1a] Difference from control test is significant

[2] NS

[3] NS

[4] Regression of dose on mortality is significant

LC_{30} = 507 mgs./kg.
LC_{50} = 1078 mgs./kg.
LC_{70} = 2293 mgs./kg.
LC_{90} = 4758 mgs./kg.

[5] NS - F (Cal) <= F (.05)

Table 28

DATA SUMMARY

Stannous Chloride in Prop Glycol
via Yolk at 96 Hrs.

Dose of Compound Injected (mgs./kg.)	(mgs./egg)	Number of Eggs	Percent Mortality[4]	Percent Abnormal Chicks Hatched[5]	Percent H-S-V-L Abnormalities
Control	None	438	6.84	6.39	1.59
Solvent	None	110	9.09	9.09	1.81
150.0	7.5	109	44.03[1]	19.26[2]	6.42
300.0	15.0	109	44.03[1]	16.51	8.25[3]
600.0	30.0	109	56.88[1]	23.85[2a]	7.33[3]
900.0	45.0	110	50.00[1]	27.27[2a]	10.00[3]
1200.0	60.0	110	53.63[1]	38.18[2a]	10.00[3]

1 Difference from control test is highly significant
2 Difference from control group response is significant
2a Difference from control group response is highly significant
3 Difference from control group response is significant
4 [illegible] F (Cal) ≤ F (.05)
5 Regression of dose on abnormal chicks is significant

LC_{30} - 1477 mgs./kg.
LC_{50} = 5271 mgs./kg.
LC_{70} = 18,825 mgs./kg.
LC_{90} = 118,156 mgs./kg.