

# MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE  
PUBLIC HEALTH SERVICE  
FOOD AND DRUG ADMINISTRATION

TO : GRAS Review Branch, HFF-335

DATE: August 6, 1976

Dr. Herbert Blumenthal, Acting Director  
Division of Toxicology, HFF-150

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SUBJECT: Investigation of the Toxic and Teratogenic Effects of GRAS Substance  
to the Developing Chicken Embryo.

Attached is the report of the inhouse investigations of Sodium Acetate  
in the developing chicken embryo.

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

Protocol:

Sodium Acetate (1) was tested for toxic and teratogenic effects to the developing chicken embryo under four sets of conditions. It was administered in 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 fifteen or more eggs were treated under these four conditions at several dose levels until a total of seventy-five to one hundred eggs per level was reached for all levels allowing some to hatch. Groups of comparable size were treated with the solvent at corresponding volumes and untreated controls were also included in each experiment.

After treatment, all eggs were candled daily and non-viable embryos 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. All abnormalities were tabulated.

Results:

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

Columns 1 and 2 give the dose administered in milligrams per egg and milligrams per kilogram, 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., total non-viable divided by total treated eggs.

Column 5 is the total number of abnormal birds expressed as a percentage of the total eggs treated. This includes all abnormalities observed and also toxic responses such as edema, hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia or other nerve disorders.

Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton expressed as percentage of the total 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 have 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 of columns 4, 5 and 6 have 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:

Sodium acetate displayed no toxicity when injected into the air cell up to 200 mg/kg (10 mg/egg). Injection via the yolk at 0 hours resulted in an LD<sub>50</sub> of 91.509 mg/kg (4.575 mg/egg), while treatment at 96 hours gave a line whose slope was not significantly different from zero.

Scattered abnormalities were observed for all four test conditions, but in no instance were the serious abnormalities higher in incidence than or different from those observed in the background. Sodium acetate displayed no teratogenicity under the test conditions employed.

1. Sodium Acetate, Mallinckrodt, Lot # WCHS.
2. McLaughlin, J., Jr., Marliac, J. P., Verrett, M. Jacqueline, Mutchler, Mary K., and Fitzhugh, O. G., (1963) Toxicol. Appl. Pharmacol. 5, 760-770.
3. Verrett, M. J., Marliac, J. P., and McLaughlin, J., Jr., (1964) JAOAC 47, 1002-1006.
4. Finney, D. J., (1964) Probit Analysis, 2nd Ed., Cambridge Press, Cambridge, Appendix I.

Table 1

SODIUM ACETATE  
Air Cell at 0 Hours

Dose		Number of Eggs	**Percent Mortality	Total	Percent Abnormal	
mg/egg	mg/kg				Structural	
10.00	200.00	145	33.10	2.75	1.37	
5.00	100.00	145	33.10	4.82	1.37	
2.50	50.00	145	31.72	4.82	3.44	
1.250	25.00	145	32.41	2.75	0.68	
0.500	10.00	145	26.20	2.75	0.00	
Water		170	31.17	2.35	0.58	
Controls		475	15.57	1.68	1.68	

\*\*Slope not significantly different from zero  $p = 0.05$

Table 2

SODIUM ACETATE  
Air Cell at 96 Hours

Dose mg/egg	mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal	
				Total	Structural
5.00	100.00	129	37.20*	5.42*	0.77
2.50	50.00	130	35.38	10.00*	3.07
1.250	25.00	130	26.15	6.92*	0.76
0.6250	12.50	130	31.53	4.61*	2.30
0.250	5.00	130	38.46*	3.84	0.76
Water		135	24.44	0.00	0.00
Controls		475	15.57	1.68	1.68

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

\*\*Slope is negative

Table 3

SODIUM ACETATE  
Yolk at 0 Hours

Dose mg/egg	mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal	
				Total	Structural
10.00	200.00	155	73.50*	1.29	0.64
5.00	100.00	155	61.93*	0.64	0.00
2.50	50.00	155	58.06*	1.93	0.00
1.250	25.00	155	55.48*	0.64	0.00
0.500	10.00	155	49.03*	1.29	0.64
Water		140	27.85	1.42	0.71
Controls		475	15.57	1.68	1.68

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

\*\*LD<sub>50</sub> 91.509 mg/kg (4.575 mg/egg)

Table 4

SODIUM ACETATE  
Yolk at 96 Hours

Dose mg/egg	mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal	
				Total	Structural
5.00	100.00	110	52.72*	4.54	0.90
2.50	50.00	110	52.72*	0.00	0.00
1.250	25.00	109	55.04*	1.83	0.91
0.6250	12.50	110	54.54*	4.54	0.90
0.250	5.00	110	46.36*	3.63	2.72
Water		110	24.54	1.81	0.90
Controls		475	15.57	1.68	1.68

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

\*\*Slope not significantly different from zero  $p = 0.05$