Invesitgation of the Toxic & Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo-Report of the in-house investigation of **Monoammonium Glutamate** in the developing chicken embryo **9/9/76**

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MEMORANDUM

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

TO : GRAS Review Branch, HFF-335

DATE: September 9, 1976

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

FROM : M. Jacqueline Verrett, Ph.D.
Reproductive Physiology Branch, HFF-157

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

Attached is the report of the inhouse investigations of Monoammonium Glutamate in the developing chicken embryo.

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

Protocol:

Monoammonium Glutamate (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 gave 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:

Monoammonium glutamate administered via the air cell displayed low toxicity up to 200 mg/kg and no LD_{50}s could be determined. Yolk treatment at 0 hours gave a calculated LD_{50} of 15.79 mg/kg (0.79 mg/egg). At 96 hours yolk treatment resulted in a line whose slope was not significantly different from zero ($p = 0.05$).

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. Monoammonium glutamate displayed no teratogenicity under the test conditions employed.

1. Monoammonium Glutamate, Stauffer Chem. Co.; Lot No. 063074.

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

MONOAMMONIUM GLUTAMATE

Air Cell at 0 Hours

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
10.00	200.00	110	52.72	0.90	0.00
5.00	100.00	110	32.72	1.81	0.00
2.50	50.00	110	20.00*	2.72	0.00
1.250	25.00	110	16.36*	1.81	0.00
0.500	10.00	110	18.18*	1.81	0.00
Water		110	40.00	0.00	0.00
Controls		402	11.94	1.49	0.99

*Significantly different from solvent $p \leq 0.05$

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

Table 2

MONOAMMONIUM GLUTAMATE

Air Cell at 96 Hours

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
5.00	100.00	110	36.36*	4.54	3.63
2.50	50.00	109	15.59	3.66	4.58
1.250	25.00	110	13.63	1.81	1.81
0.6250	12.50	110	17.27	4.54	0.90
0.250	5.00	109	18.34	0.91	0.00
Water		110	15.45	0.90	0.00
Controls		402	11.94	1.49	0.99

*Significantly different from solvent $p \leq 0.05$

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

Table 3

MONOAMMONIUM GLUTAMATE

Yolk at 0 Hours

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
10.00	200.00	110	78.18*	0.90	0.00
5.00	100.00	110	71.81*	1.81	0.00
2.50	50.00	109	67.88*	0.00	0.00
1.250	25.00	110	62.72*	1.81	1.81
0.500	10.00	110	59.09*	0.00	0.00
Water		130	23.07	0.76	0.00
Controls		402	11.94	1.49	0.99

*Significantly different from solvent $p \leq 0.05$

**LD_{50} 15.7972 mg/kg (0.7898 mg/egg)

Table 4

MONOAMMONIUM GLUTAMATE

Yolk at 96 Hours

Dose mg/egg	Dose mg/kg Eggs	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
5.00	100.00	110	57.27*	7.27	4.54
2.50	50.00	108	47.22*	6.48	2.77
1.250	25.00	110	54.54*	6.36	3.63
0.6250	12.50	110	49.09*	4.54	4.54
0.250	5.00	110	46.36*	1.81	0.90
Water		110	29.09	1.81	1.81
Controls		402	11.94	1.49	0.99

*Significantly different from solvent $p \leq 0.05$

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