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REPORT TO

MONSANTO COMPANY

TOXICITY, REPRODUCTION AND RESIDUE STUDY WITH MCS 1016 (CP 9826) IN WHITE LEGHORN CHICKENS

JUNE 20, 1972

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MONSANTO COMPANY

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I. Introduction

At the request of Monsanto Company, a toxicity, reproduction and residue study with MCS 1016 in White Leghorn chickens was initiated. This report presents the results of the investigation conducted at Wedge's Creek Research Farm, Industrial BIO-TEST Laboratories, Inc.

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II. Summary

A toxicity, reproduction and residue study was conducted in White Leghorn chickens with MCS 1016. The levels fed were 1 (T-I), 3 (T-II) and 10 (T-III) ppm in the diet. The results of the study are as follows:

The body weights and food consumption of adult birds were normal. Six females died; one in the control group, two in the T-I group, one in the T-II group and two in the T-III group. There were no significant gross pathological changes in any of the birds.

No abnormalities or systemic signs of toxicity attributable to the ingestion of MCS 1016 were observed during the investigation.

Egg production and quality in all groups, including controls, were normal. The hatchability of eggs in the T-II group (61.4%) was lower than the hatchability in the control group (85.0%) or the other two test groups (T-I - 84.9%, T-III - 83.7%).

Specific gravity and shell thickness of eggs revealed no significant differences between the control and three test groups.

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The body weight and viability data for chicks from the control group and three test groups compared favorably. No gross pathologic changes were noted in any of the chicks at the time of sacrifice.

Respectfully submitted,

INDUSTRIAL BIO-TEST LABORATORIES, INC.

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III. Procedure

A. Experimental Animals

The animals employed in the study were White Leghorn chickens. A total of 80 hens and 16 roosters was used.

B. Organization of Groups

Ninety-six chickens were divided into one control group (C) and three test groups (T-I, T-II and T-III). A structural outline of the experiment is presented in Table I.

TABLE I

TEST MATERIAL: MCS 1016

Toxicity, Reproduction and Residue Study - White Leghorn Chickens

Outline of Experiment

Group	Number of Animals Male	Female	Dose Level (ppm)
Control	4	20	None
T-I	4	20	1.0
T-II	4	20	3.0
T-III	4	20	10.0

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C. Housing and Feeding

All groups received the same basal feed*. Test diets were prepared weekly. The test materials were diluted with acetone before addition to the feed to assure even distribution throughout the ration. An equal amount of acetone, without test material, was added to the control feed and the feed blended in the same manner as the test feeds.

All animals were allowed water and their respective control or test feed ad libitum. Dosing began when the birds were 25 weeks of age and was continued for 16 weeks when hatching results were available.

D. Observations

Observations were made daily to ascertain the presence or absence of clinical signs of toxicity indicative of test material effect. All chickens were individually weighed and the group food consumption was determined weekly. As each group began laying, the eggs were collected daily and the egg weights recorded. When the egg weights were consistently between 50 and 60 grams and at least 50 percent of the hens in the group were laying, egg collection for that group was started. The time required to meet these criteria was recorded for each group.

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The eggs were collected for the following parameters:

1. Egg Production and Quality

Eggs were collected, weighed and candled for a number of seven-day periods. The total number, the individual weights of eggs collected and the total number of uncracked and unblemished eggs were recorded.

2. Hatchability

Three-fourths of all non-defective eggs were randomly selected for hatching. These eggs were turned twice daily during the seven-day collection period, then placed in an incubator maintained at 99° to 100°F, with a wet bulb reading of 85°. This procedure was followed until a minimum of 100 eggs from the control group and each test group had been set for hatching. The following were recorded:

a. Number hatched.
b. Number unhatched.
c. Viability of chicks over a 30-day period, during which period feed and water were permitted ad libitum.
d. Examination of unhatched eggs for fertility and stage of development.
e. Weight of chicks after Day 1 and Day 30.

3. Physical Characteristics of Eggs

One-fourth of the non-defective eggs and all of the defective eggs were examined to determine the following:

a. Specific gravity
b. Thickness of shells at equatorial circumference (three measurements/egg)

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4. Residue Studies

The non-defective eggs examined for physical characteristics were quick-frozen and shipped to Monsanto Company for residue analysis (shells and frozen contents separated).

After hatching results were available, one-half of the chickens in each group were sacrificed, defeathered and subjected to a gross pathological examination. Individual samples of muscle, liver, fat and kidney from each group were collected and shipped to Monsanto Company for analysis.

The remaining chickens were fed the base diet without test material for 30 days. The eggs were collected, weighed and candled during the recovery period. Ten eggs (minimum) per group per week were quick-frozen and shipped to Monsanto Company for analysis. At the end of the recovery regimen, the remaining chickens were sacrificed and handled in the same manner as at the end of the test feeding period.

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IV. Results

A. Parental Generation

1. Body Weight Data

The average male and female body weights during the test diet period are shown in Table II. The average male and female body weights during the recovery period are shown in Table III. These data are summarized Table IV. Body weights were considered normal for all test groups when compared to the control group.

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TABLE II

TEST MATERIAL: MCS 1016

Toxicity, Reproduction and Residue Study - White Leghorn Chickens

Test Diet Period Body Weight Data

		Mean Body Weight in Kilograms Week:																
	Sex	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	M	2.05	2.06	2.09	2.12	2.14	2.16	2.16	2.18	2.20	2.22	2.22	2.25	2.26	2.27	2.25	2.26	2.26
	F	1.71	1.71	1.73	1.76	1.76	1.79	1.84	1.84	1.84	1.84	1.90	1.93	1.93	1.95	1.95	1.94	1.87
	M	2.17	2.18	2.19	2.22	2.25	2.27	2.30	2.28	2.30	2.35	2.36	2.37	2.40	2.42	2.38	2.38	2.38
	F	1.81	1.82	1.85	1.85	1.88	1.88	1.93	1.93	1.96	1.97	1.99	2.06	2.02	2.03	2.00	2.06	1.95
	M	2.08	2.15	2.20	2.24	2.29	2.28	2.32	2.35	2.39	2.45	2.47	2.50	2.57	2.56	2.56	2.58	2.58
	F	1.66	1.68	1.71	1.71	1.72	1.76	1.77	1.79	1.80	1.83	1.83	1.84	1.85	1.87	1.85	1.88	1.85
	M	1.96	1.93	2.02	2.04	2.09	2.15	2.17	2.18	2.23	2.21	2.22	2.24	2.24	2.25	2.26	2.25	2.24
	F	1.64	1.66	1.68	1.73	1.74	1.75	1.77	1.77	1.82	1.81	1.83	1.85	1.85	1.86	1.88	1.90	1.85

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TABLE III

TEST MATERIAL: MCS 1016

Toxicity, Reproduction and Residue Study - White Leghorn Chickens

Recovery Period Body Weight Data

		Mean Body Weight in Kilograms Week:			
Group	Sex	1	2	3	4
C	M	2.26	2.23	2.24	2.25
	F	1.86	1.89	1.91	1.92
T-I	M	2.26	2.17	2.19	2.25
	F	1.94	1.93	1.91	1.94
T-II	M	2.64	2.63	2.59	2.71
	F	1.85	1.82	1.83	1.84
T-III	M	2.04	2.03	2.01	2.02
	F	1.84	1.83	1.82	1.84

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TABLE IV

TEST MATERIAL: MCS 1016

Toxicity, Reproduction and Residue Study - White Leghorn Chickens

Summary of Mean Body Weight Data (Kilograms)

	Pretest		Test Period		Sacrifice	
Group	Male	Female	Male	Female	Male	Female
C	2.05	1.71	2.18	1.84	2.25	1.92
T-I	2.17	1.81	2.31	1.94	2.25	1.94
T-II	2.08	1.66	2.39	1.79	2.71	1.86
T-III	1.96	1.64	2.16	1.79	2.02	1.84

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2. Food Consumption

The food consumption data are presented in Table V. The food consumption data were considered normal for all test groups when compared to the control group.

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TABLE V

TEST MATERIAL: MCS 1016

Toxicity, Reproduction and Residue Study - White Leghorn Chickens

Average Food Consumption (grams/bird/day)

Week	Group C	T-I	T-II	T-III
		Test Period		
1	148.0	136.0	125.0	117.0
2	141.0	135.0	110.0	104.0
3	173.0	162.0	148.0	152.0
4	166.0	177.0	154.0	145.0
5	183.0	198.0	180.0	142.0
6	166.0	216.0	213.0	194.0
7	156.0	161.0	134.0	175.0
8	166.0	229.0	156.0	175.0
9	166.0	138.0	145.0	133.0
10	218.0	202.0	211.0	177.0
11	211.0	219.0	218.0	151.0
12	213.0	209.0	214.0	164.0
13	226.0	170.0	216.0	205.0
14	201.0	156.0	190.0	168.0
15	149.0	152.0	181.0	154.0
16	130.0	128.0	132.0	137.0
		Recovery Period		
1	110.0	128.0	120.0	111.0
2	142.0	132.0	138.0	158.0
3	128.0	132.0	124.0	144.0
4*	135.0	150.0	123.0	125.0

* Figured on a 9 day basis.

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3. Mortality and Reactions

No abnormalities attributable to feeding MCS 1016 or systematic signs of toxicity were observed in any of the test groups when compared to the control group.

Mortality, chronology and diagnoses are presented in Tables VI and VII.

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TABLE VI

TEST MATERIAL: MCS 1016

Toxicity, Reproduction and Residue Study - White Leghorn Chickens

Mortality, Chronology and Diagnoses

Group	Animal Number	Sex	Week Died	Clinical Diagnoses
C	440	F	9	Lymphomatosis
T-I	438	F	16	Lymphomatosis
T-I	483	F	16	Prolasped Rectum
T-II	441	F	5	Lymphomatosis
T-III	467	F	2	Unknown
T-III	470	F	8	Lymphomatosis

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TABLE VII

TEST MATERIAL: MCS 1016

Toxicity, Reproduction and Residue Study - White Leghorn Chickens

Mortality Data

Group	Number Tested M	Number Tested F	Number Dead M	Number Dead F	Total Dead / Total Tested	Percent Dead
C	4	20	0	1	1/24	4.2
T-I	4	20	0	2	2/24	8.3
T-II	4	20	0	1	1/24	4.2
T-III	4	20	0	2	2/24	8.3

M = Male
F = Female

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4. Gross Pathology

Postmortem examination of all birds revealed no gross pathological tissue alterations attributable to effects of the test material.

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B. Eggs

1. Egg Production and Quality

The weekly egg production data are presented in Table VIII. The age of the hens in all groups when collection was started was 30 weeks and 36 weeks when hatching collection ended.

The egg quality data are presented in Table IX.

Egg production and quality were considered normal for all test groups when compared to the control group.

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TABLE VIII

TEST MATERIAL: MCS 1016

Toxicity, Reproduction and Residue Study - White Leghorn Chickens

Egg Production Data

	Control		T-I		T-II		T-III	
	Average Weight (grams)	Percent Production/Day	Average Weight (grams)	Percent Production/Day	Average Weight (grams)	Percent Production/Day	Average Weight (grams)	Percent Production/Day
				Test Period				
1	52.8	62.8	51.5	64.2	50.5	57.1	51.2	64.2
2	53.9	75.0	53.8	80.0	52.6	73.7	53.1	75.0
3	54.6	69.2	54.7	85.0	52.0	62.1	54.6	61.4
4	55.8	70.0	55.4	77.1	54.2	62.1	55.5	81.2
5	56.7	75.7	56.3	75.0	55.4	67.8	55.6	69.9
6	57.0	66.4	56.7	72.8	55.4	71.4	55.9	81.2
7	57.4	62.8	57.2	71.4	55.8	60.9	56.1	78.9
8	58.3	63.5	58.0	71.4	55.6	64.6	56.5	78.1
9	58.6	72.8	58.6	72.1	57.1	68.4	57.6	84.1
10	59.2	66.9	58.8	70.7	57.7	66.9	58.1	73.0
11	60.5	68.9	59.7	68.1	58.2	59.8	58.0	68.0
12	59.4	56.5	60.3	67.5	58.7	64.0	57.7	66.6
13	60.6	57.1	60.1	65.0	58.5	65.4	57.9	74.6
14	60.6	58.6	60.5	55.7	58.5	54.8	58.9	69.0
15	59.8	45.8	60.9	61.4	57.1	54.1	59.2	75.3
16	59.9	46.6	60.4	46.4	57.4	48.8	58.9	66.6
				Recovery Period				
1	58.6	65.4	57.8	55.0	57.5	51.6	58.0	60.1
2	58.5	68.2	55.7	73.2	57.8	61.9	58.9	71.4
3	58.5	68.2	57.0	58.9	57.8	69.8	58.1	80.3
4*	59.4	71.0	57.3	76.0	57.8	72.0	57.3	67.5

* Figured on a [illegible]y basis.

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TABLE IX

TEST MATERIAL: MCS 1016

Toxicity, Reproduction and Residue Study - White Leghorn Chickens

Egg Quality Data

Group	Defective Eggs Number	Defective Eggs Percent	Normal Eggs Number	Normal Eggs Percent	Total Number
C	869	53.4	760	46.6	1629
T-I	961	55.8	762	44.2	1723
T-II	743	47.6	818	52.4	1561
T-III	810	46.6	929	53.4	1739

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2. Hatchability

The hatchability data are presented in Table X. The percent of eggs hatched was lower in group T-II due to greater infertility. The percent of eggs hatched in groups T-I and T-III was considered normal when compared to the control group.

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TABLE X

TEST MATERIAL: MCS 1016

Tocicity, Reproduction and Residue Study - White Leghorn Chickens

Hatchability Data

Group	Eggs Set	Eggs Hatched	Percent Hatched	Eggs Unhatched	Stages of Incubation: Full Term	Mid-Term	One Week	Infertile	Percent* Infertile
C	114	97	85.0	17	10	1	2	4	3.5
T-I	113	96	84.9	17	10	1	2	4	3.5
T-II	114	70	61.4	44	6	5	4	29	25.4
T-III	123	103	83.7	20	8	3	4	5	4.1

* Percent of total set.

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3. Specific Gravity

The specific gravity data of the eggs collected and candled are presented in Table XI. Test group data were considered normal when compared to the control group.

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TABLE XI

TEST MATERIAL: MCS 1016

Toxicity, Reproduction and Residue Study - White Leghorn Chickens

Specific Gravity Data

	Normal Eggs		Defective Eggs		Total Eggs	
Group	Number	Average Specific Gravity	Number	Average Specific Gravity	Number	Average Specific Gravity
C	221	1.078	869	1.074	1090	1.076
T-I	216	1.077	961	1.073	1177	1.075
T-II	232	1.078	743	1.074	975	1.076
T-III	254	1.081	810	1.077	1064	1.079

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4. Egg Shell Thickness

The shell thickness data are presented in Table XII. The shell thickness was considered normal for all test groups when compared to the control group.

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TABLE XII

TEST MATERIAL: MCS 1016

Toxicity, Reproduction and Residue Study - White Leghorn Chickens

Egg Shell Thickness (inches)

	Normal Eggs		Defective Eggs		Total Eggs	
Group	Number	Average Thickness (10^{-3})	Number	Average Thickness (10^{-3})	Number	Average Thickness (10^{-3})
C	221	0.013	869	0.012	1090	0.012
T-I	216	0.012	961	0.012	1177	0.012
T-II	232	0.012	743	0.012	975	0.012
T-III	254	0.013	810	0.013	1064	0.013

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C. F1 Generation

1. Mean Body Weights and Viability

The mean body weights and viability data are presented in Table XIII. Body weight data and viability data were considered normal for all test groups when compared to the control group.

2. Gross Pathology

Postmortem examination of all birds revealed no gross pathological tissue alterations.

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