REPORT FILE

Industrial BIO-TEST Laboratories, Inc.
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

REPORT TO

MONSANTO COMPANY

90-DAY SUBACUTE ORAL TOXICITY STUDY WITH MCS 1016 IN ALBINO RATS

APRIL 28, 1972

IBT NO. B9688

DSW 036893

STLCOPCB4020717

Industrial BIO-TEST Laboratories, Inc.
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

April 28, 1972

Mr. Elmer P. Wheeler
Monsanto Company
800 North Lindbergh Boulevard
St. Louis, Missouri 63166

Dear Mr. Wheeler:

Re: IBT No. B9888 - 90-Day Subacute Oral
Toxicity Study with MCS 1016 in Albino Rats

We are submitting herewith our laboratory report dated April 28, 1972, prepared in connection with the above study.

Very truly yours,

J. C. Calandra
President

JCC/kjl

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REPORT TO

MONSANTO COMPANY

90-DAY SUBACUTE ORAL TOXICITY STUDY WITH
MCS 1016
IN ALBINC RATS

APRIL 28, 1972

IBT NO. B9888

I. Introduction

A sample identified as MCS 1016 was received from the Monsanto Company for the purpose of conducting a 90-day subacute oral toxicity study using albino rats as test animals. The following report presents the results of this investigation.

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II. Summary

Ninety days of feeding MCS 1016 to groups of albino rats at dietary levels of 30, 100 and 300 ppm revealed significant elevations of liver weights and ratios for female rats fed 300 ppm. The liver weights and ratios of male rats fed 300 ppm and of all rats fed 30 and 100 ppm were not significantly elevated. No abnormalities were noted in any of the following parameters:

Body Weight Gain
Food Consumption
Survival
Hematologic, Clinical Blood Chemistry and Urologic Studies
Gross and Microscopic Pathologic Studies

Respectfully submitted,

INDUSTRIAL BIO-TEST LABORATORIES, INC.

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III. Procedure

A. Experimental Animals

The animals employed in the study were Charles River strain* albino rats. One hundred and twenty rats (60 males and 60 females) were selected for the experiment and housed individually in standard, wire-bottomed steel rat cages. Each cage bore a color-coded card identifying the animal with respect to project number, dietary level assignment, individual animal number and sex.

B. Organization of Groups

A structural outline of the experiment is shown in Table I.

TABLE I

TEST MATERIAL: MCS 1016

90-Day Subacute Oral Toxicity Study - Albino Rats

Outline of Experiment

Group	Number of Animals Males	Females	Dietary Level (ppm)
Control	15	15	None Administered
T-I	15	15	30
T-II	15	15	100
T-III	15	15	300

* Charles River Breeding Laboratories, Inc., North Wilmington, Mass.

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C. Body Weights

Each animal used in the study was weighed on the first day of the test and at weekly intervals thereafter. The weights were recorded and served as an index to growth. Weight gains were computed at the conclusion of the 90-day test period and the data subjected to statistical analyses.

D. Food Consumption and Diet Preparation

Food consumption data were collected individually for five rats of each sex in every group weekly during the study and the data recorded.

The diet for any given group was prepared by blending the appropriate amount of MCS 1016 with standard rat ration in a Hobart Mixer.

Fresh diets were prepared each week. Each rat was offered an amount of diet sufficient for one week's ad libitum feeding. However, checks were made periodically to ensure that the food jars were not empty.

E. Mortality and Reactions

Abnormal reactions and deaths were recorded daily during the investigation.

F. Hematologic, Clinical Blood Chemistry Studies and Urine Analyses

Blood and urine samples collected individually from ten rats of each sex from both the control and T-III groups after 45 and 84 days of feeding were analyzed for the following:

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1. Hematologic Studies

   a. Hematocrit Value
   b. Erythrocyte Count
   c. Hemoglobin Concentration
   d. Total Leukocyte Count
   e. Differential Leukocyte Count

2. Clinical Blood Chemistry Studies

   a. Blood Urea Nitrogen (BUN) Concentration
   b. Serum Alkaline Phosphatase (SAP) Activity
   c. Serum Glutamic-Pyruvic Transaminase (SGPT) Activity
   d. Fasted Blood Glucose Concentration

3. Urine Analyses

   a. Glucose Concentration
   b. Albumin Concentration
   c. Microscopic Elements Examination
   d. pH
   e. Specific Gravity

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C. Pathologic Studies

Following 90 days of feeding, all surviving rats were sacrificed by carbon dioxide asphyxiation and autopsied. Animals which died during the study were examined grossly unless examination was precluded by postmortem autolysis. At the time of gross examination a complete set of organs and other tissues was removed from each rat and preserved in formalin solution. Also at autopsy the weights of the liver, kidneys, spleen, gonads, heart and brain of each rat were determined and recorded.

Microscopic examination of tissues taken from ten rats of each sex from both the control and T-III groups was conducted. The following tissues, stained with Hematoxylin-Eosin, were included: esophagus, stomach (cardia, fundus and pylorus), small intestine (duodenum, jejunum and ileum), cecum, colon, liver, kidneys, spleen, pancreas, urinary bladder, pituitary gland, adrenal gland, testes, seminal vesicle, ovary, bone marrow, thyroid gland, parathyroid gland, salivary gland, prostate gland, heart, aorta, lung, lymph node (cervical and mesenteric), skeletal muscle, peripheral nerve, bone (femur), spinal cord, uterus, trachea, eye, optic nerve and brain (cerebrum, cerebellum and pons).

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H. Organ Weights, Organ to Body Weight and Organ to Brain Weight Ratios

Statistical analyses were conducted upon the absolute organ weights and their corresponding ratios to the weight of the body and brain. An Analysis of Variance was conducted first and any significant effects disclosed by that treatment were further studied by "t"-tests.

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IV. Results

A. Body Weights

Body weight data collected during the 90-day test period are summarized in Table II. Also included in the table are 90-day average total weight gains.

Statistical comparisons of final body weights and total weight gains revealed no significant differences between test and control rats.

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TABLE II

TEST MATERIAL: MCS 1016

90-Day Subacute Oral Toxicity Study - Albino Rats

Body Weight and Total Weight Gain Data

Summary of Mean Values

[illegible] Level (ppm)	Sex	Body Weight (grams) Week: 0	1	2	3	4	5	6	7	8	9	10	11	12	13	Total Weight Gain (grams/rat)
[illegible]	M	77	167	207	255	294	317	338	360	387	397	403	419	431	452	375
	F	85	141	164	190	204	211	218	236	243	251	253	256	263	269	184
30	M	82	142	170	198	221	241	259	291	304	341	367	387	410	421	339
	F	90	143	168	199	211	219	228	243	255	260	260	272	273	285	205
300	M	86	166	204	254	294	309	325	353	381	390	418	419	422	440	354
	F	86	142	169	195	210	216	226	242	246	250	258	268	264	279	193
[illegible]	M	82	167	202	262	297	317	340	365	387	399	415	428	427	454	372
	F	86	152	171	201	215	222	230	243	232	256	238	252	246	269	183

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B. Food Consumption

Food consumption data collected during the 90-day test period are summarized in Table III.

Test rats ate amounts of food equal to that consumed by control rats.

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TABLE III

TEST MATERIAL: MCS 1016

90-Day Subacute Oral Toxicity Study - Albino Rats

Food Consumption Data

Summary of Mean Values

Dietary Level (ppm)	Sex	Food Consumption (grams/rat/seven days) Week: 1	2	3	4	5	6	7	8	9	10	11	12	13	Total Food Consumption (grams/rat)
Control	M	88	92	131	144	161	170	144	158	163	159	179	185	165	1939
	F	86	98	118	122	113	134	93	133	132	130	172	146	145	1622
30	M	83	129	142	144	150	182	172	156	162	181	151	167	187	2006
	F	78	98	108	113	153	149	126	117	125	111	144	145	140	1607
100	M	132	130	155	151	126	187	194	183	182	150	190	182	174	2139
	F	99	140	117	118	173	182	116	118	130	116	144	128	139	1720
300	M	70	140	150	180	161	175	156	198	188	163	186	187	175	2126
	F	141	126	110	123	135	133	108	135	163	118	138	128	130	1688

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C. Mortality and Reactions

Seven deaths occurred during the study. Two of these deaths were ascribed to an acute respiratory infection, while the other five resulted from trauma incurred during the collection of blood samples.

No untoward behavioral reactions were noted among any of the animals employed in the study.

D. Hematologic Studies

The results of the hematologic studies conducted on blood samples taken from ten rats of each sex in the control and T-III groups after 45 and 84 days of feeding are summarized in Tables IV and V.

No outstanding differences between test and control rats were noted with respect to any of the parameters investigated.

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TABLE IV

TEST MATERIAL: MCS 1016

90-Day Subacute Oral Toxicity Study - Albino Rats

Hematologic Data

Summary of Mean Values

Dietary Level (ppm)	Sex	Total Leukocyte Count (thousands/mm^3) Day: 45	84	Erythrocyte Count (millions/mm^3) Day: 45	84	Hemoglobin Concentration (g/100 ml) Day: 45	84	Hematocrit Value (percent) Day: 45	84
Control	M	20.8	20.8	7.85	8.46	15.4	15.1	40.6	41.4
	F	17.4	14.5	7.59	7.79	14.9	14.7	38.7	40.0
200	M	21.7	22.3	7.86	8.70	15.3	15.7	40.1	43.4
	F	14.4	16.2	7.91	7.91	15.4	15.0	40.8	41.6

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TABLE V

TEST MATERIAL: MCS 1016

90-Day Subacute Oral Toxicity Study - Albino Rats

Hematologic Data

Summary of Mean Values

Dietary Level (ppm)	Sex	Differential Leukocyte Count (Number of Cells per Hundred)									
		Lymphocytes Day:		Neutrophils Day:		Monocytes Day:		Eosinophils Day:		Basophils Day:	
		45	84	45	84	45	84	45	84	45	84
Control	M	92.8	85.8	5.0	10.6	1.6	3.2	0.6	0.7	0.0	0.0
	F	90.6	85.7	7.6	11.7	1.8	2.1	0.0	0.5	0.0	0.0
500	M	90.8	85.7	8.4	11.5	0.8	2.2	0.0	0.6	0.0	0.0
	F	85.4	86.3	12.6	10.8	1.0	2.2	1.0	1.8	0.0	0.0

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B. Clinical Blood Chemistry Studies

The results of the clinical chemistry studies conducted on blood samples obtained from ten rats of each sex in the control and T-III groups after 45 and 84 days of feeding are summarized in Tables VI and VII.

Values for test rats were not different from those of control rats.

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TABLE VI

TEST MATERIAL: MCS 1016

90-Day Subacute Oral Toxicity Study - Albino Rats

Clinical Blood Chemistry Data

Summary of Mean Values

Dietary Level (ppm)	Sex	Serum Alkaline Phosphatase Activity (King-Armstrong Units) Day: 45	84	Serum Glutamic-Pyruvic Transaminase Activity (Dade Units) Day: 45	84
Control	M	27	25	41	31
	F	18	18	46	31
300	M	39	19	37	38
	F	21	14	29	27

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TABLE VII

TEST MATERIAL: MCS 1016

90-Day Subacute Oral Toxicity Study - Albino Rats

Clinical Blood Chemistry Data

Summary of Mean Values

Dietary Level (ppm)	Sex	Blood Urea Nitrogen Concentration (mgs %) Day: 45	84	Fasted Blood Glucose Concentration (mgs %) Day: 45	84
Control	M	13	17	113	134
	F	16	19	118	116
300	M	14	19	110	126
	F	12	17	102	115

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F. Urine Analyses

The results of the periodic examinations of urine specimens collected from ten rats of each sex in the control and T-III groups after 45 and 84 days of feeding are summarized in Table VIII.

No significant differences between the urine of test rats and control rats were observed.

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TABLE VIII

TEST MATERIAL: MCS 1016

90-Day Subacute Oral Toxicity Study - Albino Rats

Urine Analysis Data

Summary of Mean Values

[illegible]ry Level [illegible]	Sex	Glucose Day: 45	Glucose Day: 84	Albumin Day: 45	Albumin Day: 84	Microscopic Elements Day: 45	Microscopic Elements Day: 84	pH Day: 45	pH Day: 84	Specific Gravity Day: 45	Specific Gravity Day: 84
[illegible]ntrol	M	n	n	t	t	N	+2	6.8	8.0	1.015	1.031
	F	n	n	t	t	N	+3	7.0	8.1	1.016	1.024
[illegible]	M	n	n	n	t	N	N	6.8	6.6	1.020	1.036
	F	n	n	n	n	N	N	7.0	6.6	1.018	1.028

[illegible] and Albumin

[illegible]egative
[illegible]; less than 30 mg/100 ml urine

[illegible]scopic Elements

[illegible] = normal
[illegible] = trace; less than 5 per low power field
[illegible] = 5-10 per low power field
[illegible] = 10-15 per low power field
[illegible] = more than 15 per low power field

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C. Pathologic Studies.

1. Gross Pathologic Findings.

No outstanding differences were noted between test and control rats upon gross pathological examination.

2. Organ Weight and Organ to Body and Organ to Brain Weight Ratio Data

The results of the statistical analyses conducted on absolute organ weights, organ to body weight and organ to brain weight ratios are summarized in Tables IX through XIV.

Significant differences between a test group and the control group are designated by asterisks following the test values.

Significant elevations of liver weights and ratios were noted for female rats fed 300 ppm. The liver weights and ratios of male rats fed 300 ppm and of all rats fed 30 and 100 ppm were not significantly elevated.

The other statistically significant intergroup differences which were noted were considered to be normal for a random population of albino rats. The lack of any consistent dose or sex related response and the absence of any deleterious histopathologic changes further substantiate that none of the intergroup differences were related to the ingestion of MCS 1016.

DSW 036914

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TABLE IX

TEST MATERIAL: MCS 1016

90-Day Subacute Oral Toxicity Study - Albino Rats

Organ Weight and Ratio Data

Summary of Mean Values

Organ: Liver

[illegible] Level	Organ Weight (g)		Organ/Body Weight Ratio (g/100 g)		Organ/Brain Weight Ratio (g/g)	
	Males	Females	Males	Females	Males	Females
[illegible]	15.2	8.7	3.36	3.21	7.73	4.73
[illegible]	15.1	8.6	3.61	3.01*	7.56	4.55
[illegible]	14.4	9.1	3.28	3.28	7.33	4.77
[illegible]	15.9	10.4*	3.53	3.87**	8.18	5.64**

*Statistically significant difference at the 95 percent confidence level.
**Statistically significant difference at the 99 percent confidence level.

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TABLE X

TEST MATERIAL: MCS 1016

90-Day Subacute Oral Toxicity Study - Albino Rats

Organ Weight and Ratio Data

Summary of Mean Values

Organ: Kidney

[illegible] Level	Organ Weight (g)		Organ/Body Weight Ratio (g/100 g)		Organ/Brain Weight Ratio (g/g)	
	Males	Females	Males	Females	Males	Females
[illegible]	3.02	1.91	0.668	0.706	1.53	1.09
[illegible]	2.89	1.95	0.692	0.686	1.44	1.08
[illegible]	3.15	2.05	0.715	0.742	1.60	1.08
[illegible]	2.96	2.05	0.655	0.760	1.50	1.11

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TABLE XI

TEST MATERIAL: MCS 1016

90-Day Subacute Oral Toxicity Study - Albino Rats

Organ Weight and Ratio Data

Summary of Mean Values

Organ: Spleen

[illegible] Level	Organ Weight (g)		Organ/Body Weight Ratio (g/100 g)		Organ/Brain Weight Ratio (g/g)	
	Males	Females	Males	Females	Males	Females
[illegible]	0.85	0.55	0.187	0.204	0.429	0.282
25	0.86	0.58	0.209	0.204	0.427	0.303
100	0.79	0.60	0.179	0.218	0.402	0.318
400	0.83	0.60	0.181	0.223	0.428	0.328

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TABLE XII

TEST MATERIAL: MCS 1016

90-Day Subacute Oral Toxicity Study - Albino Rats

Organ Weight and Ratio Data

Summary of Mean Values

Organ: Gonads

[illegible] Level	Organ Weight (g)		Organ/Body Weight Ratio (g/100 g)		Organ/Brain Weight Ratio (g/g)	
[illegible]	Males	Females	Males	Females	Males	Females
	3.19	0.135	0.708	0.0501	1.61	[illegible]
	3.37	0.104	0.807**	0.0367**	1.67	[illegible]
	3.36	0.117	0.769*	0.0423*	1.70	[illegible]
	3.34	0.121	0.736	0.0449	1.69	[illegible]

* Statistically significant difference at the 95 percent confidence level.
** Statistically significant difference at the 99 percent confidence level.

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TABLE XIII

TEST MATERIAL: MCS 1016

90-Day Subacute Oral Toxicity Study - Albino Rats

Organ Weight and Ratio Data

Summary of Mean Values

Organ: Heart

Dose Level (ppm)	Organ Weight (g)		Organ/Body Weight Ratio (g/100 g)		Organ/Brain Weight Ratio (g/g)	
	Males	Females	Males	Females	Males	Females
Control	1.43	0.98	0.318	0.361	0.727	0.501
20	1.35	1.02	0.323	0.359	0.674	0.508
200	1.49	0.98	0.340	0.353	0.757	0.519
2000	1.41	1.00	0.312	0.370	0.717	0.508

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TABLE XIV

TEST MATERIAL: MCS 1016

90-Day Subacute Oral Toxicity Study - Albino Rats

Organ Weight and Ratio Data

Summary of Mean Values

Organ: Brain

Dietary Level (ppm)	Organ Weight (g)		Organ/Body Weight Ratio (g/100 g)	
	Males	Females	Males	Females
Control	1.97	1.84	0.441	0.687
30	2.00	1.89	0.482	0.666
100	1.97	1.90	0.462	0.695
300	1.97	1.83	0.437	0.682

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3. Histopathologic Findings

Histopathologic examination of tissues and organs taken from ten rats of each sex in both the control and T-III groups was conducted.

Tables XV and XVI list all histopathologic changes noted.

All of the lesions noted in the microscopic examination of tissues were those of spontaneous disease and are not unusual for the albino rat. The most frequent findings were lesions in the trachea and lungs, indicating chronic murine pneumonia. These occurred in the control as well as the rats fed MCS 1016.

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IBT No. B9888
Monsanto

I have completed a histopathologic evaluation of a series of rat tissues from IBT B9888. There are no changes which can be attributed to the test material. The lesions described are those of spontaneous disease and they are not unusual for the rat.

Ward R. Richter

Ward R. Richter, D.V.M., M.S.
Diplomate, American College of
Veterinary Pathologists

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TABLE XV

TEST MATERIAL: MCS 1016

90-Day Subacute Oral Toxicity Study - Albino Rats

Histopathologic Changes

Group: Control

Number of Animals	Organ Examined	Findings	Incidence	Average Grade
Males	Trachea	Tracheitis	4	1.0
	Lung	Chronic respiratory disease	5	1.0
	Kidney	Focal interstitial nephritis	1	1.0
	Urinary bladder	Calculus	5	1.0
	Prostate	Inflammation	1	1.0
Females	Trachea	Tracheitis	2	1.0
	Lung	Chronic respiratory disease	2	1.0
	Liver	Focal hepatitis	3	1.0

All other tissues and organs, as listed on page 6, were normal histologically.

Grading System

0.5 = minimal
1.0 = slight
2.0 = mild
3.0 = moderate
4.0 = severe
5.0 = extreme

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TABLE XVI

TEST MATERIAL: MCS 1016

90-Day Subacute Oral Toxicity Study - Albino Rats

Histopathologic Changes

Group: 300 ppm

Number of Animals	Organ Examined	Findings	Incidence	Average Grade
10 Males	Trachea	Tracheitis	5	1.0
	Lung	Chronic respiratory disease	7	1.0
	Liver	Focal lymphoid infiltration	3	1.0
		Vacuolar change	1	1.0
	Colon	Parasites	1	1.0
	Kidney	Focal lymphoid infiltration	3	1.0
	Urinary bladder	Calculus	4	1.0
	Brain	Focal encephalitis (nosematosis)	1	1.0
10 Females	Trachea	Tracheitis	3	1.0
	Lung	Chronic respiratory disease	2	2.0
	Kidney	Focal lymphoid infiltration	3	1.0
	Brain	Focal encephalitis (nosematosis)	1	1.0

All other tissues and organs, as listed on page 6, were normal histologically.

Grading System
0.5 = minimal
1.0 = slight
2.0 = mild
3.0 = moderate
4.0 = severe
5.0 = extreme

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