

YOUNGER LABORATORIES

Biochemists . . . Pharmacologists . . . Analysts

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Certificate of Analysis

January 12th, 1966

SUBJECT -

Toxicological Investigation Of: MCS - 404

Nonsanto Sample Number 245

Nonsanto Project Number Y-65-214

STUDY CONDUCTED FOR -

Nonsanto Company, St. Louis, Missouri

EXPERIMENTAL PROCEDURE -

A) Oral LD₅₀ (Rats, Mixed Sex)

The undiluted compound was fed by stomach tube to Sprague-Dawley strain albino male and female rats.

After the approximate Minimum Lethal Dose was determined, groups of male and female rats were fed in increasing doses at increments of 0.1 fractional log intervals at four levels designed to blanket the toxicity range thereby supplying data for calculation of the LD₅₀ which was done according to a modification of the method of E. J. de Beer.

Observations were made for toxic symptoms and the viscera of the animals that succumbed were examined macroscopically.

The data are shown in Table I.

B) Oral MLD (Rabbits, Mixed Sex)

The undiluted compound was fed in increasing doses at increments of 0.2 fractional log intervals by stomach tube to New Zealand white male and female rabbits.

Observations were made for toxic symptoms and the viscera of the animals that succumbed were examined macroscopically.

The data are shown in Table II.

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EXPERIMENTAL PROCEDURE - (Continued)

C) Skin Absorption MLD (Rabbits, Mixed Sex)

The undiluted compound was applied in increasing doses at increments of 0.2 fractional leg intervals to the closely clipped, intact skin of New Zealand white male and female rabbits.

The treated areas were covered with plastic strips and the animals placed in wooden stocks for periods up to twenty-four hours, after which time they were assigned to individual cages.

Observations were made for toxic symptoms and the viscera of the animals that succumbed were examined macroscopically.

The data are shown in Table III.

D) Skin Irritation (Rabbits, Mixed Sex)

The undiluted compound was applied to the clipped, intact skin of male and female albino rabbits and removed after twenty-four hours. The application was covered with plastic strips to retard evaporation and avoid contamination.

Observations were made over a period of several days for irritation.

The data, scored according to the method of Draize, Woodard and Calvery (Journal of Pharm. and Exp. Therapeutics, Volume 82, December, 1944) are shown in Table IV.

E) Eye Irritation (Rabbits, Mixed Sex)

0.1 milliliter of undiluted sample was placed in the conjunctival sac of the right eye of each of three albino rabbits and observations made over a period of several days for inflammation.

The eyes were rinsed with warm isotonic saline solution after twenty-four hours.

The data, scored according to the method of Draize, et al, are shown in Table V.

F) Vapor Inhalation (Male Rats)

Four mature male rats were placed in a metal chamber of 35 liters capacity and exposed for six hours to a concentrated atmosphere of vapors produced by passing a stream of air through 100 milliliters of the compound contained in a 300-milliliter Erlenmeyer flask. Vapors from the flask passed into a one liter bottle to remove droplets and then into the chamber.

Air flow through the sample was five liters per minute as measured by a calibrated rotameter. This was sufficient to violently agitate the liquid. No supplementary air was introduced inasmuch as the above supply was ample for the animals oxygen requirements.

The animals were observed for behavior and, since there were no deaths, all were held for ten days observation.

The data are shown in Table VI.

SUMMARY -

A) Oral LD₅₀ (Rats, Mixed Sex)

The Oral LD₅₀ for male and female rats was placed at 19,800 milligrams per kilogram with lower and upper limits of 17,050 to 23,165 milligrams per kilogram.

The compound was classed as practically non-toxic by oral ingestion in male and female rats.

B) Oral MLD (Rabbits, Mixed Sex)

The Minimum Lethal Oral Dose for male and female rabbits was found to be greater than 1580 milligrams per kilogram and less than 2510 milligrams per kilogram.

The compound was classed as slightly toxic by oral ingestion in male and female rabbits.

C) Skin Absorption MLD (Rabbits, Mixed Sex)

The Minimum Lethal Dose by Skin Absorption in male and female rabbits was found to be greater than 1000 milligrams per kilogram and less than 1580 milligrams per kilogram.

The compound was classed as mildly toxic by skin absorption in male and female rabbits.

D) Skin Irritation (Rabbits, Mixed Sex)

The compound was classed as a slight skin irritant when applied undiluted to intact skin of male and female rabbits.

The average maximum score was 0.6 out of a possible 8 in twenty-four hours.

E) Eye Irritation (Rabbits, Mixed Sex)

The compound was classed as a slight eye irritant in male and female rabbits.

The average maximum score was 11.6 out of a possible 110 in one hour.

F) Vaper Inhalation (Male Rats)

All animals survived the six hour exposure as well as the following ten day observation period.

It was concluded that the vapors were relatively non-toxic under conditions of the test.

YOUNGER LABORATORIES

Fred M. Younger
BY: FRED M. YOUNGER

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T A B L E I

THE ORAL LD₅₀ OF 'MGS-404' FOR RATS

Sample Fed Undiluted

<u>Animal No. - Sex</u>	<u>Weight</u> <u>Gm.</u>	<u>Dose</u> <u>Mg./Kg.</u>	<u>Fate</u>
1- Female	230	12,600	Survived
2- Male	245	12,600	Survived
3- Female	220	12,600	Survived
4- Male	240	12,600	Survived
5- Female	225	12,600	Survived
6- Male	250	15,800	Survived
7- Female	235	15,800	Survived
8- Male	260	15,800	Survived
9- Female	240	15,800	Died
10- Male	265	15,800	Survived
11- Female	235	20,000	Died
12- Male	255	20,000	Survived
13- Female	225	20,000	Died
14- Male	250	20,000	Died
15- Female	230	20,000	Survived
16- Male	270	25,100	Died
17- Female	235	25,100	Died
18- Male	255	25,100	Survived
19- Female	240	25,100	Died
20- Male	260	25,100	Died

DISCUSSION -

The Oral LD₅₀ for male and female rats was placed at 19,800 milligrams per kilogram with lower and upper limits of 17,030 to 23,165 milligrams per kilogram.

The compound was classed as practically non-toxic by oral ingestion in male and female rats.

Survival time was several hours to four days with most deaths occurring in three to four days.

Toxic symptoms included severe diarrhea, loss of appetite, and increasing weakness.

At autopsy the gastrointestinal tract was gaseous and there was liver congestion.

T A B L E II

THE MINIMUM LETHAL ORAL DOSE OF 'MCS-404' FOR RABBITS

Sample Fed Undiluted

Animal No. - Sex	Weight Kg.	Dose Mg./Kg.	Weight Change 5 Days Later Kg.	Fate
1 - Female	2.5	1000	+ 0.2	Survived
2 - Male	2.4	1580	+ 0.1	Survived
3 - Female	2.6	2510	- 0.2	Died -- 5 Days
4 - Male	3.1	2510	- 0.3	Died -- 7 Days
5 - Female	2.7	3980	- 0.2	Died -- 8 Days
6 - Male	2.7	6310	- 0.3	Died -- 8 Days

DISCUSSION -

The Minimum Lethal Oral Dose for male and female rabbits was found to be greater than 1580 milligrams per kilogram and less than 2510 milligrams per kilogram.

The compound was classed as slightly toxic by oral ingestion in male and female rabbits.

Survival time was five to eight days.

Toxic symptoms included gradual loss of appetite with increasing weakness, lethargy, and collapse.

At autopsy there was renal, liver, and pulmonary hyperemia by macroscopic examination.

The material in this report is to be used in development of the product and may be given to various individuals, contacts, but it is not to be given to the public or the press. The purpose of this report is to inform the individuals who are in the development of the product and to inform the individuals who are in the development of the product. The purpose of this report is to inform the individuals who are in the development of the product and to inform the individuals who are in the development of the product. The purpose of this report is to inform the individuals who are in the development of the product and to inform the individuals who are in the development of the product.

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T A B L E I I I
THE MINIMUM LETHAL DOSE OF 'MCS-404'
BY SKIN ABSORPTION IN RABBITS

Sample Applied Undiluted

Animal No. - Sex	Weight K.G.	Dose Mg./Kg.	Weight Change 5 Days Later Kg.	Fate
1 - Female	2.7	631	+ 0.1	Survived
2 - Male	3.3	1000	- 0.1	Survived
3 - Female	2.5	1580	- 0.2	Died -- 6 Days
4 - Male	2.6	2510	- 0.4	Died -- 9 Days
5 - Female	2.3	3980	- 0.2	Died - 14 Days
6 - Male	2.8	6310	- 0.3	Died -- 5 Days

DISCUSSION -

The Minimum Lethal Dose by Skin Absorption in male and female rabbits was found to be greater than 1000 milligrams per kilogram and less than 1580 milligrams per kilogram.

The compound was classed as mildly toxic by skin absorption in male and female rabbits.

Survival time was five to fourteen days.

Toxic symptoms included weakness after three to six days, reduced appetite, dyspnea, collapse, and paralysis.

At autopsy there was pulmonary hyperemia; otherwise no visceral changes of consequence were noted.

T A B L E I V

SKIN IRRITATION IN RABBITS AFTER APPLICATION OF 'MCS-404'

Sample Applied Undiluted

Animal No. - Sex	1 Hour	Numerical Evaluation At The End Of			
		24 Hours	48 Hours	72 Hours	120 Hours
1 - Male	0	1	1	0	0
2 - Female	0	0	0	0	0
3 - Male	0	1	0	0	0
Average	0.0	0.6	0.3	0.0	0.0

DISCUSSION -

The compound was classed as a slight skin irritant when applied undiluted to intact skin of male and female rabbits.

The average maximum score was 0.6 out of a possible 8 in twenty-four hours.

No skin changes were noted at the end of one hour. Overnight there were two instances of very slight redness with no edema for an average score of 0.6. Within seventy-two hours all animals were free of inflammation.

T A B L E V

EYE IRRITATION IN RABBITS AFTER APPLICATION OF 'MCS-404'

Sample (0.1 Milliliter) Applied Undiluted

<u>Animal No. - Sex</u>	<u>1 Hour</u>	<u>Numerical Evaluation At The End Of</u>			
		<u>24 Hours</u>	<u>48 Hours</u>	<u>72 Hours</u>	<u>120 Hours</u>
1 - Female	11	9	4	2	0
2 - Male	11	6	2	0	0
3 - Female	13	8	4	4	0
Average	11.6	7.6	3.3	2.0	0.0

DISCUSSION -

The compound was classed as a slight eye irritant.

The average maximum score was 11.6 out of a possible 110 in one hour.

There was blinking but no pawing at the eyes following application.

Mild discharge, slight redness, very slight edema, and slight corneal dullness was recorded after one hour. Edema and discharge disappeared overnight. Within three days only a trace to slight erythema remained on two animals.

To: Monsanto Company
St. Louis, Missouri
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T A B L E VI

INHALATION OF 'MCS-404' VAPORS BY RATS

Average Temperature Inside Chamber 74° F.
Average Relative Humidity Inside Chamber 44 %
Amount of Sample -- To start 100.0 cc
Recovered 99.5 cc
Vaporized or left in
equipment 0.5 cc (0.5%)

<u>Animal No.-Sex</u>	<u>Fate</u>	<u>Observations During Exposure</u>
1 - Male	Survived	No immediate effect ...
2 - Male	Survived	Lethargy after one hour ...
3 - Male	Survived	No discomfort ...
4 - Male	Survived	Normal respiration ... No weakness.

DISCUSSION -

All animals survived the six hour exposure as well as the following ten day observation period.

It was concluded that the vapors were relatively non-toxic under conditions of the test.

With the exception of moderate lethargy, the animals showed no reaction to the vapors. Weakness was absent and no nasal or ocular inflammation was noted. Activity and appearance were normal the day following exposure and no respiratory complications developed during the ten day observation period.