YOUNGER LABORATORIES
Biochemists . . . Pharmacologists . . . Analysts

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Certificate of Analysis

January 10th, 1966

SUBJECT -

Toxicological Investigation Of: MCS 395

Monsanto Sample Number 244

Monsanto Project Number Y-65-213

STUDY CONDUCTED FOR -

Monsanto Company, St. Louis, Missouri

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EXPERIMENTAL PROCEDURE -

A) Oral LD_{50} (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 LD_{50} 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 log 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 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 55 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 500 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 -

MCS 395

A) Oral LD_{50} (Rats, Mixed Sex)

The Oral LD_{50} for male and female rats was placed at 4615 milligrams per kilogram with lower and upper limits of 4060 to 5260 milligrams per kilogram.

The compound was classed as slightly 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 2510 milligrams per kilogram and less than 3980 milligrams per kilogram.

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

D) Skin Irritation (Rabbits, Mixed Sex)

The compound was classed as non-irritating when applied undiluted to the intact skin of male and female rabbits.

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 8.3 out of a possible 110 in one hour.

F) Vapor 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 practically non-toxic under conditions of the test.

YOUNGER LABORATORIES

BY: FRED M. YOUNGER

TABLE I

THE ORAL LD_{50} OF 'MCS 395' FOR RATS

Sample Fed Undiluted

Animal No. - Sex	Weight Gm.	Dose Mg./Kg.	Fate
1- Female	225	3160	Survived
2- Male	240	3160	Survived
3- Female	220	3160	Survived
4- Male	255	3160	Survived
5- Female	235	3160	Survived
6- Male	260	3980	Survived
7- Female	230	3980	Died
8- Male	270	3980	Survived
9- Female	235	3980	Survived
10- Male	255	3980	Survived
11- Female	230	5010	Died
12- Male	255	5010	Survived
13- Female	225	5010	Died
14- Male	265	5010	Survived
15- Female	235	5010	Died
16- Male	280	6310	Died
17- Female	225	6310	Died
18- Male	245	6310	Died
19- Female	240	6310	Died
20- Male	265	6310	Died

DISCUSSION -

The Oral LD_{50} for male and female rats was placed at 4615 milligrams per kilogram with lower and upper limits of 4060 to 5260 milligrams per kilogram.

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

Survival time was several hours to three days with most deaths occurring overnight. Toxic symptoms included severe diarrhea, increasing weakness, tremors, and collapse.

At autopsy there was inflammation of the gastric mucosa and renal hyperemia macroscopically.

T A B L E II

THE MINIMUM LETHAL ORAL DOSE OF 'MCS 395' FOR RABBITS

Sample Fed Undiluted

Animal No. - Sex	Weight Kg.	Dose Mg./Kg.	Weight Change 5 Days Later Kg.	Fate
1 - Female	2.6	631	0.0	Survived
2 - Male	3.0	1000	+ 0.1	Survived
3 - Female	2.7	1580	- 0.1	Survived
4 - Male	3.1	2510	- 0.4	Died -- 8 Days
5 - Female	2.7	3980	- 0.3	Died -- 5 Days
6 - Male	2.9	6310	-----	Died -- 2 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 two to eight days.

Toxic symptoms in animals #4 and #5 included poor appetite in two to three days, weight loss, increasing weakness, tremors, and collapse.

At autopsy there was evidence of liver dysfunction and renal hyperemia.

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TABLE III

THE MINIMUM LETHAL DOSE OF 'MCS 395'
BY SKIN ABSORPTION IN RABBITS

Sample Applied Undiluted

Animal No. - Sex	Weight Kg.	Dose Mg./Kg.	Weight Change 5 Days Later Kg.	Fate
1 - Male	3.0	631	+ 0.2	Survived
2 - Female	2.5	1000	+ 0.1	Survived
3 - Male	2.8	1580	+ 0.1	Survived
4 - Female	2.4	2510	- 0.1	Survived
5 - Male	2.5	3980	- 0.3	Died - 7 Days
6 - Female	3.1	6310	- 0.2	Died - 9 Days

DISCUSSION -

The Minimum Lethal Dose by Skin Absorption in male and female rabbits was found to be greater than 2510 milligrams per kilogram and less than 3980 milligrams per kilogram.

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

Survival time was seven to nine days.

Toxic symptoms in animals #5 and #6 included reduced appetite and activity in three to four days with increasing weakness, dyspnea, and paralysis.

At autopsy the lungs were hemorrhagic and there was liver discoloration macroscopically.

T A B L E IV

SKIN IRRITATION IN RABBITS AFTER APPLICATION OF 'MCS 395'

Sample Applied Undiluted

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

DISCUSSION -

The compound was classed as non-irritating to intact rabbit skin when applied undiluted.

No edema or erythema developed on any of the animals as a result of a twenty-four hour exposure to the undiluted compound.

All animals received a score of zero throughout the observation period.

TABLE V

EYE IRRITATION IN RABBITS AFTER APPLICATION OF 'MCS 395'

Sample (0.1 Milliliter) Applied Undiluted

Animal No. - Sex	Numerical Evaluation At The End Of 1 Hour	24 Hours	48 Hours	72 Hours	120 Hours
1 - Female	7	4	2	0	0
2 - Male	7	4	4	0	0
3 - Female	11	6	4	2	0
Average	8.3	4.6	3.3	0.6	0.0

DISCUSSION -

The compound was classed as a slight eye irritant in male and female rabbits. The average maximum score was 8.3 out of a possible 110 in one hour.

Little discomfort was shown immediately following application.

After one hour there was slight discharge and redness, practically no edema, and very slight corneal dullness. Iris and cornea were clear in twenty-four hours and within three days only a trace of redness of the palpebral conjunctivae remained on one animal.

T A B L E VI

INHALATION OF 'M C S 395' VAPORS BY RATS

Average Temperature Inside Chamber ..... 76° F.

Average Relative Humidity Inside Chamber ..... 43 %

Amount of Sample -- To Start ..... 100.0 cc
Recovered ..... 99.7 cc
Vaporized or left in equipment ..... 0.3 cc (0.3%)

Animal No. - Sex	Fate	Observations During Exposure
1 - Male	Survived	No immediate reaction ...
2 - Male	Survived	Normal breathing rate ...
3 - Male	Survived	No discomfort or nasal discharge ...
4 - Male	Survived	No weakness ... Moderate lethargy after two hours.

DISCUSSION -

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

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

The animals appeared to be unaffected by the exposure. Nasal and ocular mucosae were normal when the animals were removed from the chamber.
The ten day observation period was uneventful.