YOUNGER LABORATORIES
Biochemists . . . Pharmacologists . . . Analysts

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Certificate of Analysis

November 12th, 1964

SUBJECT -

Toxicological Investigation Of: MCS 312

Monsanto Sample Number 146

Monsanto Project Number Y-64-133

STUDY CONDUCTED FOR -

Monsanto Company, St. Louis, Missouri

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) 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 II.

EXPERIMENTAL PROCEDURE - (Continued)

C) Skin Irritation (Rabbits)

The undiluted compound was applied to the clipped, intact skin of 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 III.

D) Eye Irritation (Rabbits)

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 IV.

E) Vapor Inhalation (Male Rats)

Four 150-gram male rats were placed in a glass desiccator, 250 mm in diameter, 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 250-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 V.

SUMMARY -

MCS 512

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

The Oral LD_{50} for male and female rats was placed at 6500 milligrams per kilogram with lower and upper limits of 5460 to 7735 milligrams per kilogram.

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

B) Skin Absorption MLD (Rabbits, Mixed Sex)

The Minimum Lethal Dose by Skin Absorption in male and female rabbits was found to be greater than 2000 milligrams per kilogram and less than 3160 milligrams per kilogram.

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

SUMMARY - (Continued)

C) Skin Irritation (Rabbits)

The compound was classed as a slight skin irritant when applied undiluted to intact rabbit skin.

The average maximum score was 1.3 out of a possible 8 in twenty-four hours.

D) Eye Irritation (Rabbits)

The compound was classed as a slight eye irritant.

The average maximum score was 13.0 out of a possible 110 in twenty-four hours.

E) 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

Fred M. Younger

BY: FRED M. YOUNGER

TABLE I

THE ORAL LD_{50} OF 'MCS 312' FOR RATS

Sample Fed Undiluted

Animal No. - Sex	Weight GM.	Dose MG./KG.	Fate
1- Female	195	5,010	Survived
2- Female	210	5,010	Died
3- Male	230	5,010	Survived
4- Male	225	5,010	Survived
5- Female	220	5,010	Survived
6- Female	210	6,310	Died
7- Male	240	6,310	Survived
8- Male	230	6,310	Survived
9- Female	205	6,310	Died
10- Female	215	6,310	Survived
11- Male	250	7,940	Survived
12- Male	230	7,940	Died
13- Female	215	7,940	Died
14- Female	225	7,940	Died
15- Male	245	7,940	Survived
16- Male	230	10,000	Died
17- Female	210	10,000	Died
18- Female	225	10,000	Died
19- Male	240	10,000	Died
20- Male	250	10,000	Died

DISCUSSION -

The Oral LD_{50} for male and female rats was placed at 6300 milligrams per kilogram with lower and upper limits of 5460 to 7735 milligrams per kilogram.

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

Survival time was one to four days with most deaths occurring in two days.

Toxic symptoms included severe diarrhea, tremors, dyspnea, and collapse.

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

T A B L E II

THE MINIMUM LETHAL DOSE OF 'M C S 312'
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	2.7	794	+ 0.1	Survived
2 - Female	3.1	1260	- 0.1	Survived
3 - Male	2.8	2000	- 0.3	Survived
4 - Female	2.7	3160	- 0.4	Died -- 6 Days
5 - Male	2.6	5010	- 0.4	Died -- 6 Days
6 - Female	2.8	7940	------	Died -- 4 Days

DISCUSSION -

The Minimum Lethal Dose by Skin Absorption in male and female rabbits was found to be greater than 2000 milligrams per kilogram and less than 3160 milligrams per kilogram.

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

Survival time was four to six days.

Toxic symptoms included gradual loss of appetite, increasing weakness, and tremors. Violent convulsions and squealing were noted approximately twenty-four hours before deaths.

At autopsy there were hemorrhagic areas in the lungs as well as liver discoloration and renal congestion macroscopically.

TABLE III

SKIN IRRITATION IN RABBITS AFTER APPLICATION OF 'MCS 312'

Sample Applied Undiluted

Animal Number	Numerical Evaluation At The End Of 1 Hour	24 Hours	48 Hours	72 Hours	120 Hours
1	0	1	1	0	0
2	0	2	1	1	0
3	0	1	0	0	0
Average	0.0	1.3	0.6	0.3	0.0

DISCUSSION -

The compound was classed as a slight skin irritant when applied undiluted to intact rabbit skin.

The average maximum score was 1.3 out of a possible 8 in twenty-four hours.

No skin changes were noted in one hour. Overnight there was barely perceptible redness on two animals and well-defined redness on the third animal for an average score of 1.3. No edema developed. Within seventy-two hours two animals were free of color and all received a score of zero in five days.

T A B L E IV

EYE IRRITATION IN RABBITS AFTER APPLICATION OF 'M C S 512'

Sample (0.1 Milliliter) Applied Undiluted

Animal Number	Numerical Evaluation At The End Of 1 Hour	24 Hours	48 Hours	72 Hours	120 Hours
1	12	8	4	2	0
2	15	11	6	4	0
3	12	8	4	2	0
Average	13.0	9.0	4.6	2.6	0.0

DISCUSSION -

The compound was classed as a slight eye irritant.

The average maximum score was 13.0 out of a possible 110 in twenty-four hours.

Discomfort was mild immediately following application.

Slight redness of the palpebral conjunctivae, slight discharge, a trace of edema, and very mild corneal dullness with no iritis developed within one hour. Erythema reduced in twenty-four hours and discharge ceased. Within seventy-two hours corneal clarity was normal and all animals received a score of zero in five days.

T A B L E V

INHALATION OF 'MCS 312' VAPORS BY RATS

Average Temperature Inside Chamber ..... 78° F.

Average Relative Humidity Inside Chamber ..... 62 %

Amount Of Sample — To Start ..... 100.0 cc
Recovered ..... 99.5 cc
Vaporized ..... 0.5 cc (0.5%)

Animal No. - Sex	Fate	Observations During Exposure
1 - Male	Survived	No nasal discharge ...
2 - Male	Survived	No dyspnea ...
3 - Male	Survived	Mild lethargy ...
4 - Male	Survived	No weakness or discomfort ...
		Animals appear unaffected.

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.

As mentioned in the table, the animals appeared to be unaffected by the six hour exposure. Discomfort was at a minimum and the only change in behavior was mild lethargy after approximately one hour.

The ten day observation period was uneventful.