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September 17th, 1973

Certificate of Analysis

C + 25 AM

SUBJECT -

Toxicological Investigation Of: MCS 1489 -- (RC) MIC 231499

Monsanto Project Number Y-73-158

STUDY CONDUCTED FOR -

Monsanto Company, St. Louis, Missouri

SUMMARY -

A) Single Oral Dose LD_{50} (Albino Rats)

The Single Oral Dose LD_{50} for male and female rats was placed at 8300 milligrams per kilogram with lower and upper limits of 7550 to 9130 milligrams per kilogram. The compound was classed as practically non-toxic by oral ingestion in male and female rats.

B) Acute Skin Absorption Minimal Lethal Dose (Albino Rabbits) - 24 Hours Exposure

The Acute Skin Absorption Minimal Lethal Dose for male and female rabbits was found to be greater than 3160 milligrams per kilogram and less than 5010 milligrams per kilogram.

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

C) Skin Irritation (Albino Rabbits) - 24 Hours Exposure

The compound (0.5 milliliter applied undiluted) was classed as a moderate irritant to intact skin of male and female rabbits.

The average maximum score was 3.0 out of a possible 8 in twenty-four hours.

D) Eye Irritation (Albino Rabbits) - 24 Hours Exposure

The compound (0.1 milliliter applied undiluted) was classed as a slight eye irritant in male and female rabbits.

The average maximum score was 10.0 out of a possible 110 in one hour.

E) Vapor Inhalation (Male Rats) - Ambient Temperature

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

Average concentration of vapors in chamber: 0.0 Gram / M^3.

YOUNGER LABORATORIES, INC.

BY: MELVIN D. BIRCH

MONS 044370

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EXPERIMENTAL PROCEDURE -

A) Single Oral Dose LD_{50} (Albino Rats)

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

After the approximate Minimal 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 the method of E. J. de Beer.

Observations were made for toxic signs and the viscera of the test animals were examined macroscopically.

The data are shown in Table I.

B) Acute Skin Absorption Minimal Lethal Dose (Albino Rabbits) - 24 Hours Exposure

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 held in wooden stocks for periods up to twenty-four hours, after which time they were assigned to individual cages.

Observations were made for toxic signs and the viscera of the test animals were examined macroscopically.

The data are shown in Table II.

C) Skin Irritation (Albino Rabbits) - 24 Hours Exposure

0.5 Milliliter of undiluted sample was applied to the clipped, intact skin of three New Zealand white male and female rabbits under a one inch by one inch square patch, two single layers thick. The patches were held in place with adhesive tape. The trunk of each animal was wrapped with plastic strips, to retard evaporation and avoid contamination, for the twenty-four hour exposure period.

Observations were made over a period of seven 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 (Albino Rabbits) - 24 Hours Exposure

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

The eyes were rinsed with warm isotonic saline solution after the twenty-four hour reading. The left eye served as a control.

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

E) Vapor Inhalation (Male Rats) - Ambient Temperature

Six mature male rats were placed in a stainless steel chamber of 35-liters capacity and exposed for six hours to a concentrated atmosphere of vapors produced by passing a stream of air through 83.0 grams 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 4.0 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 during exposure and for ten days following exposure. The viscera of the test animals were examined macroscopically.

The data are shown in Table V.

TABLE I

THE SINGLE ORAL DOSE LD_{50} OF
'MCS 1489 -- (RC) MIC 231499'
FOR RATS

Sample Fed Undiluted

Animal No. - Sex	Weight Gm.	Dose Mg. / Kg.	Fate
1 - Female	230	6,310	Survived
2 - Male	220	6,310	Survived
3 - Female	220	6,310	Survived
4 - Male	220	6,310	Survived
5 - Female	220	6,310	Survived
6 - Male	235	7,940	Died
7 - Female	230	7,940	Survived
8 - Male	225	7,940	Died
9 - Female	260	7,940	Survived
10 - Male	205	7,940	Survived
11 - Female	240	10,000	Died
12 - Male	205	10,000	Survived
13 - Female	215	10,000	Died
14 - Male	230	10,000	Died
15 - Female	235	10,000	Died
16 - Male	215	12,600	Died
17 - Female	245	12,600	Died
18 - Male	225	12,600	Died
19 - Female	240	12,600	Died
20 - Male	230	12,600	Died

DISCUSSION --

The Single Oral Dose LD_{50} for male and female rats was placed at 8300 milligrams per kilogram with lower and upper limits of 7550 to 9130 milligrams per kilogram.

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

Survival time was two to five days with most deaths occurring within three days.

Toxic signs included reduced appetite and activity (one to three days in survivors), increasing weakness, collapse and death.

At autopsy there was lung hyperemia, areas of liver discoloration and acute gastrointestinal inflammation.

Surviving animals were sacrificed seven days after dosing. The viscera appeared normal by macroscopic examination.

TABLE II

THE ACUTE SKIN ABSORPTION MINIMAL LETHAL DOSE OF
'MCS 1489 -- (RG) MIC 231499'
FOR RABBITS

Sample Applied Undiluted - 24 Hour Exposure

Animal No. - Sex	Weight Kg.	Dose Mg. / Kg.	Weight Change 5 Days Later Kg.	Fate
1 - Male	2.1	2000	0.0	Survived
2 - Female	2.1	3160	- 0.2	Survived
3 - Male	1.9	5010	- 0.4	Died -- 6 Days
4 - Female	2.0	7940	-----	Died -- 4 Days

DISCUSSION --

The Acute Skin Absorption Minimal Lethal Dose for male and female rabbits was found to be greater than 3160 milligrams per kilogram and less than 5010 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 signs included reduced appetite and activity (three to five days in survivors), increasing weakness, collapse and death.

At autopsy there was hemorrhagic areas of the lungs, liver discoloration, enlarged gall bladder, and gastrointestinal inflammation.

Surviving animals were sacrificed fourteen days after dosing. The viscera appeared normal by macroscopic examination.

TABLE III

SKIN IRRITATION IN RABBITS AFTER APPLICATION OF
'MCS 1489 -- (RG) MIC 231499'

Sample (0.5 Milliliter) Applied Undiluted - 24 Hour Exposure

Animal No. - Sex	Numerical Evaluation At The End Of 1 Hour	24 Hours	48 Hours	72 Hours	120 Hours	168 Hours
1 - Male	0	4	2	0	0	0
2 - Female	0	2	1	0	0	0
3 - Male	0	3	1	0	0	0
Average	0.0	3.0	1.3	0.0	0.0	0.0

DISCUSSION --

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

The average maximum score was 3.0 out of a possible 8 in twenty-four hours.

Observations Following Application --

1-Hour:	No skin changes; zero readings
24-Hours:	Very slight to slight erythema, very slight to slight edema
48-Hours:	Very slight erythema, very slight edema in one instance
72-Hours:	No erythema or edema; zero readings
120- 168-Hours:	No erythema or edema; zero readings

T A B L E IV

E Y E IRRITATION IN RABBITS AFTER APPLICATION OF
'MCS 1489 -- (RC) MIC 231499'

Sample (0.1 Milliliter) Applied Undiluted - 24 Hour Exposure

	Numerical Evaluation At The End Of					
Animal No. - Sex	1 Hour	24 Hours	48 Hours	72 Hours	120 Hours	168 Hours
1 - Male	10	4	0	0	0	0
2 - Female	10	4	2	0	0	0
3 - Male	10	4	0	0	0	0
Average	10.0	4.0	0.6	0.0	0.0	0.0

DISCUSSION --

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

The average maximum score was 10.0 out of a possible 110 in one hour.

Observations Following Application --

Immediate:	Discomfort was slight
10-Minutes:	Moderate erythema, copious discharge; no edema
1-Hour:	Moderate erythema, copious discharge; no edema
24-Hours:	Slight erythema slight discharge; no edema
48-Hours:	Slight erythema in one instance; no edema or discharge
72-Hours:	Eyes normal; zero readings
120- 168-Hours:	Eyes normal; zero readings

TABLE V

INHALATION OF

'MCS 1489 -- (RC) MIC 231499'

VAPORS BY RATS

(AMBIENT TEMPERATURE)

Average Temperature Inside Chamber ..... 80° F.

Average Relative Humidity Inside Chamber ..... 85 %

Amount of Sample -- To Start ..... 83.0 Grams
Recovered ..... 83.0 Grams
Vaporized or Left in Equipment ..... 0.0 Gram (0.0%)

Average Concentration of Vapors In Chamber..... 0.0 Gram / M^3

Animal No. - Sex	Fate	Observations During Exposure
1 - Male	Survived	No nasal or ocular discharge ...
2 - Male	Survived	
3 - Male	Survived	No weakness or drowsiness of
4 - Male	Survived	consequence ...
5 - Male	Survived	
6 - Male	Survived	Respiration appeared normal.

DISCUSSION --

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

Observations Following Exposure --

Upon removal from the chamber respiration was normal, and no other signs of toxic distress were noted.

The ten day observation period was uneventful. Weight gain was normal.

Surviving animals were sacrificed ten days after exposure. The viscera appeared normal by macroscopic examination.