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YOUNGER LABORATORIES INCORPORATED

Consulting and Analytical Services

CHEMICAL . . . MICROBIOLOGICAL . . . BIOLOGICAL

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January 12th, 1972

Certificate of Analysis

SUBJECT -

Toxicological Investigation Of: MCS 1228 German Mine Fluid --
Lot: OR 162483

Monsanto Sample Number 189

Monsanto Project Number Y-71-189

STUDY CONDUCTED FOR -

Monsanto 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 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 LD₅₀ 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 (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 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.

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EXPERIMENTAL PROCEDURE - (Continued)

C) Skin Irritation (Rabbits, Mixed Sex)

0.5 Milliliter of undiluted sample was applied to the clipped, intact skin of 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 Calvary (Journal of Pharm. and Exp. Therapeutics, Volume 82, December, 1944) are shown in Table III.

D) 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 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 Rate) - 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 153.5 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.

F) Vapor Inhalation (Male Rate) - Sample Heated to 150°C.

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 146.0 grams of the compound contained in a 300-milliliter erlenmeyer flask. The sample was maintained at a temperature of 150°C. by immersing the flask in a heated sand bath.

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 VI.

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SUMMARY -

MCS 1228 German Mine Fluid -- Lot: OR 162483

A) Oral LD₅₀ (Rats, Mixed Sex)

The Oral LD₅₀ for male and female rats was placed at 12,900 milligrams per kilogram with lower and upper limits of 11,700 to 14,230 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 (Rabbits, Mixed Sex)

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 (Rabbits, Mixed Sex)

The compound was classed as non-irritating when applied undiluted to intact skin of male and female rabbits. The average maximum score was 0.3 out of a possible 8 in twenty-four hours.

D) Eye Irritation (Rabbits, Mixed Sex)

The compound was classed as a slight eye irritant in male and female rabbits. The average maximum score was 12.6 out of a possible 110 in one hour.

E) Vapor Inhalation (Male Rats) - Ambient Temperature

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.

F) Vapor Inhalation (Male Rats) - Sample Heated to 150°C.

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.

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BY: MELVIN D. BIRCH

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TABLE I
THE ORAL LD₅₀ OF

'MCS 1228 German Mine Fluid -- Lot: OR 162483'
FOR RATS

Sample Fed Undiluted

Animal No. - Sex	Weight Gm.	Dose Mg. / Kg.	Fate
1- Female	225	7,940	Survived
2- Male	225	7,940	Survived
3- Female	225	7,940	Survived
4- Male	240	7,940	Survived
5- Female	215	7,940	Survived
6- Male	225	10,000	Survived
7- Female	205	10,000	Survived
8- Male	235	10,000	Survived
9- Female	250	10,000	Died
10- Male	215	10,000	Survived
11- Female	245	12,600	Survived
12- Male	205	12,600	Died
13- Female	225	12,600	Died
14- Male	230	12,600	Survived
15- Female	240	12,600	Survived
16- Male	205	15,800	Died
17- Female	210	15,800	Died
18- Male	220	15,800	Survived
19- Female	240	15,800	Died
20- Male	265	15,800	Died

DISCUSSION -

The Oral LD₅₀ for male and female rats was placed at 12,900 milligrams per kilogram with lower and upper limits of 11,700 to 14,230 milligrams per kilogram.

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

Survival time was four to six days.

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

At autopsy there was slight liver discoloration and 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
'MOS 1228 German Mine Fluid -- Lot: OR 162483'
FOR RABBITS

Sample Applied Undiluted

<u>Animal No. - Sex</u>	<u>Weight Kg.</u>	<u>Dose Mg./Kg.</u>	<u>Weight Change 5 Days Later Kg.</u>	<u>Fate</u>
1 - Female	2.7	1260	+ 0.1	Survived
2 - Male	2.5	2000	0.0	Survived
3 - Female	2.7	3160	- 0.4	Survived
4 - Male	2.4	5010	- 0.4	Died - 14 Days
5 - Female	2.0	7940	- 0.6	Died - 11 Days
6 - Male	2.4	7940	- 0.6	Died - 12 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 eleven to fourteen days.

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

At autopsy there was hemorrhagic areas of the lungs, discolored liver (mottled and jaundiced), enlarged gall bladder, and gastrointestinal tract was gaseous and inflamed.

Surviving animals were sacrificed fourteen days after dosing. The viscera of animals #1 and #2 appeared normal by macroscopic examination; animal #3 showed liver hyperemia.

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T A B L E III

SKIN IRRITATION IN RABBITS AFTER APPLICATION OF

'MCS 1228 German Mine Fluid-- Lot: OR 162483'

Sample (0.5 Milliliter) Applied Undiluted

Animal No. - Sex	Numerical Evaluation At The End Of					
	1 Hour	24 Hours	48 Hours	72 Hours	120 Hours	168 Hours
1 - Male	0	0	0	0	0	0
2 - Female	0	1	1	0	0	0
3 - Male	0	0	0	0	0	0
Average	0.0	0.3	0.3	0.0	0.0	0.0

DISCUSSION -

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

The average maximum score was 0.3 out of a possible 8 in twenty-four hours.

Observations following application -

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

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T A B L E I V

E Y E I R R I T A T I O N I N R A B B I T S A F T E R A P P L I C A T I O N O F

'MCS 1228 German Mine Fluid -- Lot: OR 162483'

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	168 Hours
1 - Male	12	2	0	0	0	0
2 - Female	14	2	0	0	0	0
3 - Male	12	2	0	0	0	0
Average	12.6	2.0	0.0	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 12.6 out of a possible 110 in one hour.

Observations following application --

Immediate:	Discomfort was slight
10-Minutes:	Slight erythema and edema, moderate discharge
1-Hour:	Slight to moderate erythema, slight edema, copious discharge
24-Hours:	Slight erythema; no edema or discharge
48-Hours:	No erythema, edema, or discharge; zero readings
72- 168-Hours:	No erythema, edema, or discharge; zero readings

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T A B L E V

INHALATION OF

'MCS 1228 German Mine Fluid -- Bot GR: 162483' VAPORS

BY RATS (AMBIENT TEMPERATURE)

Average Temperature Inside Chamber 74° F.
Average Relative Humidity Inside Chamber 78 %
Amount of Sample -- To start 153.5 Grams
Recovered 153.5 Grams
Vaporized or left
in equipment 0.0 Gram (0.0%)
Average Concentration of Vapors in Chamber 0.0 Gram/M³

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

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.

Observations following exposure -

Respiration was normal when animals were removed from the chamber.

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.

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TABLE VI

IMMERSION OF

'MCS 1228 German Mine Fluid -- Lot: CR 162483' VAPORS
 BY RATS (SAMPLE HEATED TO 150° F.)

Average Temperature Inside Chamber 78° F.
 Average Relative Humidity Inside Chamber 85 %
 Amount of Sample -- To start 146.0 Grams
 Recovered 140.8 Grams
 Total Vaporized 5.2 Grams (3.6 %)
 Recovered from Condenser. 0.7 Gram (0.5 %)
 Weight of vapors entering
 chamber 4.5 Grams (3.1 %)
 Average Concentration Of Vapors In Chamber .. 3.12 Grams / m³

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

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.

Observations following exposure -

Respiration was normal when animals were removed from the chamber.

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