

YOUNGER LABORATORIES

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Certificate of Analysis

February 26th, 1957

SUBJECT -

Toxicological Investigation Of:

Hydraulic Fluid OS-81 - Monsanto Sample No. 1

Hydraulic Fluid OS-83 - Monsanto Sample No. 2

Monsanto Project No. Y-57-1

TESTS CONDUCTED FOR -

Monsanto Chemical Company, St. Louis, Missouri

EXPERIMENTAL PROCEDURE -

A) Oral LD₅₀ In Rats

Each undiluted compound was introduced into the stomachs of Sprague-Dawley strain albino rats by means of a rubber catheter. After the approximate Minimum Lethal Dose was found, groups of rats were fed at levels designed to blanket the toxicity range thereby supplying data for calculation of the LD₅₀. This was done according to a modification of the method of Bliss. Observations were made for outward symptoms of toxicity and the viscera of selected animals from those succumbing was examined macroscopically.

The data are shown in Table I and Table II.

B) The Minimum Lethal Oral Dose For Rabbits

New Zealand white rabbits were fed orally varying amounts of test compound. The viscera of animals that succumbed was examined macroscopically.

The data are found in Table III and Table IV.

C) Skin Irritation In Rabbits

Undiluted sample was applied to the closely clipped, intact skin of albino rabbits and observations made over a period of several days for irritation.

The results, based on the scoring technique of Draize, Woodard and Calvery (J. of Pharm. and Exp. Therapeutics, Vol. 82, No. 4, Dec. 1944), are shown in Table V and Table VI.

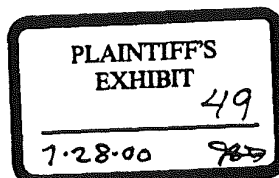
D) Eye Irritation In Rabbits

0.1 ML. of undiluted sample was placed in the conjunctival sac of the right eye of each of three albino rabbits and the degree of irritation scored according to the method of Draize, Woodard and Calvery (J. of Pharm. and Exp. Therapeutics, Vol. 82, No. 4, Dec. 1944).

The data are found in Table VII and Table VIII.

MET 005491

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EXPERIMENTAL PROCEDURE - (Continued)

E) Vapor Inhalation In Rats

Four mature male rats were placed in a metal chamber of approximately 50 liters capacity and exposed for six hours to an atmosphere of hydraulic fluid vapors produced by aspirating the compound at room temperature. The liquid was poured into a three hole, round bottom flask and compressed air passed over an orifice, the lower end of which was immersed in the liquid. A glass tube led from the flask into the chamber. A glass impinger was placed in the line to break up droplets and allow, as nearly as practicable, only vapors to pass. The animals were observed for behavior and, since there were no deaths, all rats were held for two weeks additional observation.

The data are shown in Table IX and Table X.

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TABLE I
 THE ORAL LD₅₀ OF 'HYDRAULIC FLUID OS-31'
 FOR RATS

<u>Animal No. - Sex</u>	<u>Weight Gm.</u>	<u>Dose Gm./Kg.</u>	<u>Fate</u>
1- Female	240	6.00	Survived
2- Female	210	6.00	Died
3- Male	235	6.00	Survived
4- Female	210	6.00	Survived
5- Male	225	6.00	Survived
6- Male	245	7.50	Died
7- Female	215	7.50	Survived
8- Female	200	7.50	Survived
9- Female	220	7.50	Survived
10- Male	250	7.50	Died
11- Male	210	7.50	Survived
12- Female	190	8.25	Died
13- Female	195	8.25	Survived
14- Female	215	8.25	Survived
15- Male	230	8.25	Survived
16- Male	255	8.25	Died
17- Female	200	8.25	Survived
18- Male	240	9.00	Died
19- Female	195	9.00	Died
20- Female	205	9.00	Survived
21- Male	235	9.00	Survived
22- Female	215	9.00	Died

DISCUSSION -

The Oral LD₅₀ was found to be 8.60 grams per kilogram with fiducial limits of 6.12 to 9.05 grams per kilogram. Survival time was over nine to four days. There was diarrhea, irritability, and nervousness observed. Some survivors lost as much as 30% of their starting weight before regaining normal appetite.

At autopsy there was liver damage, moderate kidney congestion, and gastrointestinal inflammation.

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TABLE II
 THE ORAL LD₅₀ OF 'HYDRAFLUO FLUID CS-63'
 FOR RATS

<u>Animal No. - Sex</u>	<u>Weight Gm.</u>	<u>Dose Gm. / Kg.</u>	<u>Fate</u>
1- Female	210	27.5	Survived
2- Female	220	27.5	Survived
3- Female	205	27.5	Died
4- Male	240	27.5	Survived
5- Male	210	27.5	Survived
6- Female	195	30.0	Died
7- Female	205	30.0	Survived
8- Female	215	30.0	Survived
9- Male	230	30.0	Died
10- Male	250	30.0	Survived
11- Female	190	35.0	Survived
12- Female	200	35.0	Died
13- Male	225	35.0	Died
14- Female	185	35.0	Survived
15- Female	195	35.0	Died
16- Female	215	35.0	Survived
17- Male	240	40.0	Died
18- Male	255	40.0	Survived
19- Female	230	40.0	Died
20- Female	210	40.0	Died
21- Female	205	40.0	Died

DISCUSSION -

The Oral LD₅₀ was placed at 34.3 grams per kilogram with lower and upper limits of 32.6 to 38.5 grams per kilogram. Survival time was two to seven days. Severe diarrhea, trembling, irritability, and lethargy developed. Weight losses ran to 25% of starting weight.

At autopsy the stomach was gaseous and irritated. The liver was discolored and hyperemic. There was also pulmonary congestion.

MET 005494

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T A B L E III
 THE MINIMUM LETHAL ORAL DOSE OF 'HYDRAULIC FLUID CS-81'
 FOR RABBITS

<u>Animal No. - Sex</u>	<u>Weight Kg.</u>	<u>Dose Gm./Kg.</u>	<u>Weight Change 4 Days After Dosing</u>	<u>Fate</u>
1 - Male	2.8	0.25	0.0 %	Survived
2 - Female	1.9	0.35	0.0 %	Survived
3 - Male	1.9	0.50	-15.0 %	Died—4 Days
4 - Male	2.3	0.75	-10.0 %	Died—4 Days
5 - Male	2.1	1.00	-13.0 %	Died—7 Days
6 - Female	1.8	1.50	-12.0 %	Died—4 Days
7 - Female	1.8	2.00	-20.0 %	Died—5 Days
8 - Male	2.3	3.00	-13.0 %	Died—5 Days
9 - Male	2.3	5.00	-15.0 %	Died—5 Days

DISCUSSION -

The Minimum Lethal Dose was in the range of 0.35 to 0.50 gram per kilogram. Survival time was four to seven days. The animals gradually became weak from loss of appetite. There appeared to be partial paralysis after two to three days.

At autopsy liver and spleen were congested. There appeared to be blood changes.

T A B L E IV
 THE MINIMUM LETHAL ORAL DOSE OF 'HYDRAULIC FLUID CS-83'
 FOR RABBITS

<u>Animal No. - Sex</u>	<u>Weight Kg.</u>	<u>Dose Gm./Kg.</u>	<u>Weight Change 4 Days After Dosing</u>	<u>Fate</u>
1 - Male	2.6	2.25	0.0 %	Survived
2 - Female	2.1	3.00	+ 4.0 %	Survived
3 - Male	2.4	3.50	0.0 %	Survived
4 - Female	1.9	4.25	- 8.0 %	Survived
5 - Male	2.4	5.00	-13.0 %	Died—5 Days
6 - Male	2.2	6.50	-10.0 %	Died—6 Days

DISCUSSION -

The Minimum Lethal Dose was 4.25 to 5.00 grams per kilogram. Survival time was five to six days. Weight losses resulted from poor appetite. Animals No. 5 and No. 6 gradually became lethargic but did not appear to develop paralysis.

At autopsy kidneys, liver, and lungs were all hyperemic.

MET 005495

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T A B L E V
SKIN IRRITATION IN RABBITS AFTER APPLICATION OF
'HYDRAULIC FLUID OS-81'

<u>Animal No.</u>	<u>Numerical Evaluation At The End Of</u>			
	<u>2 Hours</u>	<u>24 Hours</u>	<u>48 Hours</u>	<u>72 Hours</u>
1	1	1	1	0
2	0	1	0	0
3	1	1	0	0
Average	0.6	1.0	0.3	0.0

DISCUSSION -

After two hours, two rabbits showed barely perceptible redness for an average score of 0.6 out of a possible 8.0. No edema developed. Overnite very slight erythema was observed on all animals. The residue was removed from the skin after twenty-four hours. Two of the animals were given a zero score on the second day.

Hydraulic Fluid OS-81 was found to be a rather mild skin irritant.

T A B L E VI
SKIN IRRITATION IN RABBITS AFTER APPLICATION OF
'HYDRAULIC FLUID OS-83'

<u>Animal No.</u>	<u>Numerical Evaluation At The End Of</u>			
	<u>2 Hours</u>	<u>24 Hours</u>	<u>48 Hours</u>	<u>72 Hours</u>
1	0	1	0	0
2	1	1	1	0
3	0	1	0	0
Average	0.3	1.0	0.3	0.0

DISCUSSION -

The average score after two hours was 0.3, consisting of very slight erythema on one animal. All rabbits showed barely perceptible redness in twenty-four hours at which time the remaining compound was wiped off the skin. Two rabbits were found to be free of irritation within forty-eight hours.

Hydraulic Fluid OS-83 was classed as a rather mild skin irritant.

MET 005496

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T A B L E VII
EYE IRRITATION IN RABBITS AFTER APPLICATION OF
'HYDRAULIC FLUID OS-81'

<u>Animal No.</u>	<u>Numerical Evaluation At The End Of</u>				
	<u>1 Hour</u>	<u>24 Hours</u>	<u>48 Hours</u>	<u>72 Hours</u>	<u>120 Hours</u>
1	8	6	2	2	0
2	10	6	4	2	0
3	6	4	2	0	0
Average	8.0	5.3	2.6	1.3	0.0

DISCUSSION -

There was slight discomfort shown immediately upon application. The average score after one hour was 8.0 out of a possible 110.0. Moderate erythema and lacrimation with slight edema was observed. The iris was practically unaffected. Overnight lacrimation returned to normal and the average score reduced to 5.3. Two of the animals had a slight degree of erythema remaining after seventy-two hours. Hydraulic Fluid OS-81 was classed as a mild eye irritant.

T A B L E VIII
EYE IRRITATION IN RABBITS AFTER APPLICATION OF
'HYDRAULIC FLUID OS-83'

<u>Animal No.</u>	<u>Numerical Evaluation At The End Of</u>				
	<u>1 Hour</u>	<u>24 Hours</u>	<u>48 Hours</u>	<u>72 Hours</u>	<u>120 Hours</u>
1	8	6	4	2	0
2	12	8	4	2	0
3	10	6	4	2	0
Average	10.0	6.6	4.0	2.0	0.0

DISCUSSION -

The degree of irritation was quite similar for both Hydraulic Fluid OS-81 and Hydraulic Fluid OS-83. Mild discomfort developed immediately upon application of Hydraulic Fluid OS-83. After one hour the average score was 10.0 out of a possible 110.0. Moderate erythema of the palpebral conjunctivae, considerable lacrimation, and mild edema developed. The iris remained clear and the cornea showed little change. All animals still showed slight redness after seventy-two hours but all were free of inflammation by the fifth day.

Hydraulic Fluid OS-83 was classed as a mild eye irritant.

MET 005497

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To: Monsanto Chemical Company,
St. Louis, Missouri

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T A B L E IX
INHALATION OF 'HYDRAULIC FLUID CS-81' VAPORS
BY RATS

Average Temperature Inside Chamber 75.5° F.
Average Relative Humidity Inside Chamber 60.0 %

<u>Animal No.</u>	<u>Fate</u>	<u>Observations During Exposure</u>
1	Survived	Animals continue to move about—
2	Survived	No pawing at nose or eyes—
3	Survived	Breathing steady.
4	Survived	

DISCUSSION -

No deaths occurred during the six hours exposure nor during the subsequent two weeks observation period. Breathing and activity in general did not change materially leading to the belief that the vapors did not cause serious discomfort. Mucous membranes of the nose and throat showed only slight inflammation after exposure. Salivation was not excessive.

The fluid was agitated quite briskly at ten pounds pressure per square inch. However, little or no haze developed in the chamber atmosphere. It was possible that saturation was reached although it was apparent that the compound does not readily vaporize.

MET 005498

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T A B L E X
INHALATION OF 'HYDRAULIC FLUID CS-63' VAPORS
BY RATS

Average Temperature Inside Chamber 73.9° F.
Average Relative Humidity Inside Chamber 57.8 %

<u>Animal No.</u>	<u>Fate</u>	<u>Observations During Exposure</u>
1	Survived	Animals show only slight discomfort.
2	Survived	Continue to explore cage.
3	Survived	
4	Survived	

DISCUSSION -

The animals survived the exposure without incident. The behavior pattern was very similar to Hydraulic Fluid CS-81. No excessive lacrimation or salivation was noted. Activity was not materially reduced. Inflammation of the nose and throat was very mild to mild.

Air pressure of ten to twelve pounds per square inch agitated the fluid vigorously but apparently droplets were not carried over into the chamber. The fur of the animals remained dry. As with Hydraulic Fluid CS-81 very little vapor has accumulated in the chamber.

The vapors of both Hydraulic Fluids were considered to be only mildly irritating to rats.

MET 005499

004751

to: Monsanto Chemical Company,
St. Louis, Missouri

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SUMMARY -

Toxicological Investigation Of two new Hydraulic Fluids produced the following results:

HYDRAULIC FLUID OS-81

Oral LD₅₀ (Rats) — 8.60 Grams per Kilogram (fiducial limits 8.12 to 9.03 Gm./Kg.).

Minimum Lethal Oral Dose (Rabbits) — 0.35 to 0.50 Gram per Kilogram.

Skin Irritation (Rabbits) — Rather Mild Skin Irritant:

Average Maximum Score - 1.0 out of possible 8.0.

Eye Irritation (Rabbits) — Mild Eye Irritant:

Average Maximum Score - 8.0 out of possible 110.0.

Vapor Inhalation (Rats) — No Deaths, Mild Effect.

HYDRAULIC FLUID OS-83

Oral LD₅₀ (Rats) — 34.3 Grams Per Kilogram (fiducial limits 32.6 to 36.5 Gm./Kg.).

Minimum Lethal Oral Dose (Rabbits) — 4.25 to 5.00 Grams Per Kilogram.

Skin Irritation (Rabbits) — Rather Mild Skin Irritant:

Average Maximum Score - 1.0 out of possible 8.0.

Eye Irritation (Rabbits) — Mild Eye Irritant:

Average Maximum Score - 10.0 out of possible 110.0.

Vapor Inhalation (Rats) — No Deaths, Mild Effect.

YOUNGER LABORATORIES

Fred M. Younger
BY: FRED M. YOUNGER

TO: MONSANTO CHEMICAL COMPANY
FROM: YOUNGER LABORATORIES
SUBJECT: CERTIFICATE OF ANALYSIS
DATE: 2/25/57
ANALYST: FRED M. YOUNGER
CHECKED BY: [illegible]
APPROVED BY: [illegible]
LABORATORY: [illegible]
ST. LOUIS, MISSOURI

MET 005500

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