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Certificate of Analysis

January 16th, 1970

SUBJECT -

Toxicological Investigation Of: Hydraul AC-A -- Winter Grade 23

Monsanto Sample Number 132

Monsanto Project Number Y-69-148

STUDY CONDUCTED FOR -

Monsanto Company, St. Louis, Missouri

COPY

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 five 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 signs and the viscera of the test animals 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 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)

The undiluted compound was applied to the clipped, intact skin of albino male and female 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, 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 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) - Ambient Temperature

Six mature male rats were placed in a metal chamber of 35-liters capacity and exposed for six hours to a concentrated atmosphere of vapors produced by passing a stream of air through 117.0 grams of the compound contained in a 300-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.

SUMMARY -

Pydraul AC-A -- Winter Grade 28

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

The Oral LD_{50} for male and female rats was placed at 10,250 milligrams per kilogram with lower and upper limits of 8920 to 12,750 milligrams per kilogram.

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

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SUMMARY - (Cont

B) Skin Absorpt (Rabbits, Mixed Sex)

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

The compound lassed as slightly toxic by skin absorption in male and female rabbi

C) Skin Irrita abbits, Mixed Sex)

The compoun lassed as a slight to mild irritant when applied undiluted to intact s male and female rabbits.

The average im score was 2.0 out of a possible 8 in forty-eight hours.

D) Eye Irritat abbits, Mixed Sex)

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

The average mu score was 2.0 out of a possible 110 in one hour.

E) Vapor Inhala (le Rats) - Ambient Temperature

All animals vi the six hour exposure as well as the following ten day observation io

It was concled t the vapors were practically non-toxic under conditions of the test.

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BY: MELVIN D. BIRCH

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T A B L E I

THE ORAL LD_{50} OF

'Hydraul AC-A -- Winter Grade 28' FOR RATS

Sample Fed Undiluted

Animal No. - Sex	Weight Gm.	Dose Mg./Kg.	Fate
1- Male	205	6,310	Survived
2- Female	220	6,310	Survived
3- Male	205	6,310	Survived
4- Female	215	6,310	Survived
5- Male	220	6,310	Survived
6- Female	225	7,940	Died
7- Male	225	7,940	Survived
8- Female	240	7,940	Survived
9- Male	230	7,940	Survived
10- Female	225	7,940	Died
11- Male	250	10,000	Survived
12- Female	225	10,000	Died
13- Male	245	10,000	Survived
14- Female	215	10,000	Died
15- Male	245	10,000	Survived
16- Female	210	12,600	Died
17- Male	250	12,600	Died
18- Female	200	12,600	Died
19- Male	225	12,600	Survived
20- Female	225	12,600	Died
21- Male	235	15,800	Survived
22- Female	205	15,800	Died
23- Male	235	15,800	Died
24- Female	210	15,800	Died
25- Male	265	15,800	Died

DISCUSSION -

The Oral LD_{50} for male and female rats was placed at 10,250 milligrams per kilogram with lower and upper limits of 8,920 to 12,750 milligrams per kilogram.

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

Survival time was twelve hours to two days.

Toxic signs included reduced appetite and activity, increasing weakness, collapse, and death. Note: Sample appeared to be slightly more toxic to females than males.

At autopsy there was slight liver discoloration, hemorrhagic lungs, and acute gastrointestinal inflammation.

Surviving animals were sacrificed nine days after dosing. Macroscopic examination revealed slight liver discoloration and areas of lung congestion on the upper dosage levels; viscera of the animals on the lower dosage levels appeared normal.

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TABLE II

THE MINIMUM LETHAL DOSE OF 'Pydraul AC-A -- Winter Grade 28'
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	2.1	1260	- 0.2	Survived
2 - Female	2.4	2000	- 0.2	Survived
3 - Male	2.5	2000	- 0.1	Survived
4 - Female	2.7	2000	- 0.2	Survived
5 - Male	2.5	3160	- 0.4	Survived
6 - Female	2.5	3160	- 0.6	Died -- 5 Days
7 - Male	2.3	3160	- 0.5	Survived
8 - Female	2.4	5010	-----	Died -- 4 Days
9 - Male	2.3	7940	- 0.5	Died -- 6 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 signs included greatly reduced appetite and activity, lethargy, mild diarrhea for three to seven days, increasinb weakness, collapse, and death. Most survivors sustained slight weight losses after fourteen days.

At autopsy there was greatly enlarged gall bladder, hemorrhagic lungs, slight liver discoloration, and acute gastrointestinal inflammation.

Surviving animals were sacrificed fourteen days after dosing. Macroscopic examination revealed areas of lung congestion and slightly enlarged gall bladder in some cases.

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T A B L E III

SKIN IRRITATION IN RABBITS AFTER APPLICATION OF

'Pydraul AC-A -- Winter Grade 28'

Sample Applied Undiluted

Animal No. - Sex	Numerical Evaluation At The End Of					
	1 Hour	24 Hours	48 Hours	72 Hours	120 Hours	168 Hours
1 - Female	0	1	2	1	0	0
2 - Female	0	1	2	1	0	0
3 - Male	0	1	2	1	0	0
Average	0.0	1.0	2.0	1.0	0.0	0.0

DISCUSSION -

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

The average maximum score was 2.0 out of a possible 8 in forty-eight hours.

Observations following application --

1-Hour:	No skin changes; zero reading
24-Hours:	Barely perceptible erythema; no edema
48-Hours:	Well-defined erythema; no edema
72-Hours:	Barely perceptible erythema; no edema
120-Hours:	Skin normal; zero reading
168-Hours:	Skin normal; zero reading

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T A B L E IV

EYE IRRITATION IN RABBITS AFTER APPLICATION OF
'Pydraul AC-A -- Winter Grade 28'

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	2	0	0	0	0	0
2 - Female	2	0	0	0	0	0
3 - Male	2	0	0	0	0	0
Average	2.0	0.0	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 2.0 out of a possible 110 in one hour.

Observations following application --

Immediate:	Mild discomfort
10-Minutes:	Slight erythema, moderate discharge; no edema
1-Hour:	Slight erythema; no edema or discharge There was no corneal cloudiness and iris reaction to light was normal
24-Hours:	Eyes normal; zero reading
48- 168-Hours:	Eyes normal; zero reading

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TABLE V

INHALATION OF 'Pydraul AC-A -- Winter Grade 28'
VAPORS BY RATS

Average Temperature Inside Chamber ..... 74° F.

Average Relative Humidity Inside Chamber ..... 52 %

Amount of Sample -- To start ..... 117.0 Grams
Recovered ..... 117.0 Grams
Vaporized or left in equipment ..... 0.0 Gram (0 %)

Animal No. - Sex	Fate	Observations During Exposure
1 - Male	Survived	No immediate effect ...
2 - Male	Survived	Normal respiration ...
3 - Male	Survived	No nasal or ocular discharge ...
4 - Male	Survived	No weakness or drowsiness of consequence.
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 during and following exposure --

Immediate: No effect

1- 6-Hours: No apparent effect

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

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

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