YOUNGER LABORATORIES
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Certificate of Analysis

August 11th, 1964

SUBJECT -

Toxicological Investigation Of: Pydraul 280

Monsanto Sample Number 102

Monsanto Project Number Y-64-91

STUDY CONDUCTED FOR -

Monsanto Company, St. Louis, Missouri

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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 by intubating 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.

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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 - 350° F. (Male Rats)

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 150-milliliters of the compound contained in a 300-milliliter Erlenmeyer flask placed in a sand bath on a hot plate and the contents maintained at a temperature of 350° F.

Vapors from the flask passed into a train of two one-liter bottles 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 -

Pydraul 280

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

The Oral LD_{50} for male and female rats was placed at 11,750 milligrams per kilogram with lower and upper limits of 9,570 to 14,390 milligrams per kilogram.

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

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SUMMARY - (Continued)

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 1260 milligrams per kilogram and less than 2000 milligrams per kilogram.

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

C) Skin Irritation (Rabbits)

The compound was classed as a moderate skin irritant.

The average maximum score was 3.3 out of a possible 8 in twenty-four hours.

D) Eye Irritation (Rabbits)

The compound was classed as a moderate eye irritant.

The average maximum score was 31.3 out of a possible 110 in twenty-four hours.

E) Vapor Inhalation - 350° F. (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 mildly toxic under conditions of the test.

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BY: FRED M. YOUNGER

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TABLE I

THE ORAL LD_{50} OF 'Pydraul 280' FOR RATS

Sample Fed Undiluted

Animal No. - Sex	Weight Gm.	Dose Mg./Kg.	Fate
1- Female	220	7,940	Survived
2- Female	230	7,940	Survived
3- Male	245	7,940	Survived
4- Male	235	7,940	Died
5- Female	225	7,940	Survived
6- Female	250	10,000	Survived
7- Male	250	10,000	Survived
8- Male	245	10,000	Survived
9- Female	215	10,000	Survived
10- Female	235	10,000	Died
11- Male	250	12,600	Survived
12- Male	260	12,600	Died
13- Female	225	12,600	Died
14- Female	210	12,600	Died
15- Male	240	12,600	Survived
16- Male	255	15,800	Died
17- Female	220	15,800	Died
18- Female	225	15,800	Died
19- Male	240	15,800	Survived
20- Male	250	15,800	Died

DISCUSSION -

The Oral LD_{50} for male and female rats was placed at 11,750 milligrams per kilogram with lower and upper limits of 9,570 to 14,390 milligrams per kilogram.

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

Survival time was several hours to six days with most deaths occurring in three to four days.

Toxic symptoms included loss of appetite, severe diarrhea, increasing weakness, and collapse.

At autopsy there was renal and liver hyperemia as well as inflammation of the gastric mucosa.

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TABLE II

THE MINIMUM LETHAL DOSE OF 'Pydraul 280' 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	1,260	- 0.1	Survived
2 - Female	2.3	2,000	- 0.2	Died -- 8 Days
3 - Male	3.0	3,160	- 0.4	Died -- 7 Days
4 - Female	2.4	5,010	- 0.3	Died -- 8 Days
5 - Male	2.6	7,940	- 0.4	Died -- 5 Days
6 - Female	2.8	12,600	- 0.5	Died -- 7 Days

DISCUSSION -

The Minimum Lethal Dose by Skin Absorption in male and female rabbits was found to be greater than 1260 milligrams per kilogram and less than 2000 milligrams per kilogram.

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

Survival time was five to eight days.

Toxic symptoms included reduced appetite, weakness in two to three days, and muscular impairment particularly noticeable in the neck muscles causing the head to droop.

At autopsy there was liver discoloration and pulmonary hyperemia macroscopically.

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T A B L E III

SKIN IRRITATION IN RABBITS AFTER APPLICATION OF 'Pydraul 280'

Sample Applied Undiluted

Animal Number	Numerical Evaluation At The End Of 1 Hour	24 Hours	48 Hours	72 Hours	120 Hours	168 Hours
1	1	3	2	2	0	0
2	1	3	3	2	1	0
3	2	4	3	2	1	0
Average	1.3	3.3	2.6	2.0	0.6	0.0

DISCUSSION -

The compound was classed as a moderate skin irritant.

The average maximum score was 3.3 out of a possible 8 in twenty-four hours.

After one hour there was barely perceptible to well-defined redness with no edema. Well-defined to moderate erythema with very slight edema in twenty-four hours produced an average score of 3.3. Following removal of the compound the average score reduced to 0.6 in five days at which time barely perceptible erythema was still present on two animals.

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TABLE IV

EYE IRRITATION IN RABBITS AFTER APPLICATION OF 'Pydraul 280'

Sample (0.1 Milliliter) Applied Undiluted

Animal Number	Numerical Evaluation At The End Of 1 Hour	24 Hours	48 Hours	72 Hours	120 Hours	168 Hours
1	35	29	21	15	9	4
2	33	25	18	11	4	2
3	26	19	12	8	4	0
Average	31.3	24.3	17.0	11.3	5.6	2.0

DISCUSSION -

The compound was classed as a moderate eye irritant.

The average maximum score was 31.3 out of a possible 110 in twenty-four hours.

Considerable discomfort developed following administration.

Slight edema and discharge, moderate erythema of the palpebral conjunctivae, and mild iris congestion and corneal dullness were noted within an hour. Edema and discharge disappeared overnight. Improvement continued following irrigation leading to an average score of 2.0 in seven days.

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TABLE V

INHALATION OF 'Pydraul 280' VAPORS BY RATS
(350° F.)

Average Temperature Inside Chamber ..... 79° F.

Average Relative Humidity Inside Chamber ..... 53 %

Amount Of Sample -- To Start ..... 150.0 cc
Recovered ..... 132.0 cc
Vaporized or left in apparatus ..... 7.5 cc (5%)

Condensate in 1st liter bottle ..... 10.5 cc
Condensate in 2nd liter bottle ..... 0.0 cc

Animal No. - Sex	Fate	Observations During Exposure
1 - Male	Survived	Moderate discomfort in thirty minutes ..
2 - Male	Survived	
3 - Male	Survived	Occasional irregular breathing ..
4 - Male	Survived	Moderate nasal discharge ...
5 - Male	Survived	
6 - Male	Survived	Some weakness but no danger of collapse.

DISCUSSION -

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

It was concluded that the vapors were mildly toxic under conditions of the test.

A temperature of 350° F. was reached in approximately twenty minutes. The blue color changed to pale green and gradually turned to light brown in four hours. A moderate degree of haze was present in the chamber throughout the test. Discomfort increased with time. Moderately severe inflammation of nasal and ocular mucosae was noted when the animals were removed. All had a mild degree of bronchial rales. There was no gasping but breathing was somewhat difficult.

Inflammation reduced in twenty-four hours and within three to four days breathing was nearly normal. The animals appeared in good condition at the end of the ten day observation period.

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