PROJECT NO. Y-63-11
REPORT FILE

79

YOUNGER LABORATORIES

Biochemists . . . Pharmacologists . . . Analysts

128 CLIFF CAVE ROAD
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Certificate of Analysis

March 4th, 1963

SUBJECT -

Toxicological Investigation Of: Pyranol 1470

Monsanto Sample Number 21

Monsanto Project Number Y-63-11

STUDY CONDUCTED FOR -

Monsanto Chemical Company, St. Louis, Missouri

EXPERIMENTAL PROCEDURE -

A) Oral LD_{50} (Rats)

The undiluted compound was fed by stomach tube to Sprague-Dawley strain albino 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 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)

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 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.

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 (Rats)

Four 150-gram male rats were placed in a glass desiccator, 250 mm in diameter, and exposed for six hours to a concentrated atmosphere of vapors produced by passing a stream of air through 100 milliliters of the compound contained in a 250-milliliter tall form gas washing bottle with fritted disc. Vapors from the bottle passed into a one liter bottle to remove droplets and then into the chamber. Air flow through the sample was 6 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 -

Pyranol 1470

A) Oral LD_{50} (Rats)

The Oral LD_{50} for male and female rats was placed at 2250 milligrams per kilogram with lower and upper limits of 1990 to 2555 milligrams per kilogram.

The compound was classed as slightly toxic by oral ingestion in male and female rats.

B) Skin Absorption MLD (Rabbits)

The Minimum Lethal Dose by Skin Absorption in female rabbits was found to be greater than 2000 milligrams per kilogram and less than 3160 milligrams per kilogram. Animal #3, dosed at 2000 milligrams per kilogram, very nearly succumbed.

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

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St. Louis, Missouri
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SUMMARY - (Continued)

C) Skin Irritation (Rabbits)

The compound was classed as a moderate skin irritant.

The average maximum score was 4.6 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 32.6 out of a possible 110 in one hour.

E) Vapor Inhalation (Rats)

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

It was concluded that the vapors were slightly 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 'Pyranol 1470' FOR RATS

Sample Fed Undiluted

Animal No. - Sex	Weight Gm.	Dose Mg./Kg.	Fate
1- Female	225	1580	Survived
2- Female	210	1580	Survived
3- Male	235	1580	Survived
4- Male	250	1580	Survived
5- Female	215	1580	Survived
6- Female	220	2000	Survived
7- Male	240	2000	Survived
8- Male	245	2000	Survived
9- Female	230	2000	Died
10- Female	215	2000	Survived
11- Male	235	2510	Died
12- Male	240	2510	Survived
13- Female	220	2510	Died
14- Female	210	2510	Died
15- Male	235	2510	Died
16- Male	225	3160	Died
17- Female	220	3160	Died
18- Female	215	3160	Died
19- Male	235	3160	Died
20- Male	245	3160	Died

DISCUSSION -

The Oral LD_{50} for male and female rats was placed at 2250 milligrams per kilogram with lower and upper limits of 1990 to 2555 milligrams per kilogram.

The compound was classed as slightly toxic by oral ingestion in male and female rats.

Survival time was approximately twenty-four hours to three days with most deaths occurring in two to three days.

Toxic symptoms included increasing weakness, severe diarrhea, tremors, and collapse.

At autopsy there was inflammation of the gastric mucosa, liver discoloration, and renal hyperemia macroscopically.

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TABLE II

THE MINIMUM LETHAL DOSE OF 'Pyranol 1470'
BY SKIN ABSORPTION IN RABBITS

Sample Applied Undiluted

Animal No. - Sex	Weight Kg.	Dose Mg./Kg.	Weight Change 5 Days Later Kg.	Fate
1 - Female	2.5	794	- 0.1	Survived
2 - Female	2.2	1260	- 0.1	Survived
3 - Female	2.3	2000	- 0.2	Survived
4 - Female	2.6	3160	- 0.3	Died - 10 Days
5 - Female	2.5	5010	- 0.2	Died - 7 Days
6 - Female	2.5	7940	- 0.4	Died - 7 Days

DISCUSSION -

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

Animal #3, dosed at 2000 milligrams per kilogram, very nearly succumbed.

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

Survival time was seven to ten days.

Toxic symptoms included loss of appetite, lethargy, and increasing weakness. Weight losses ran to forty per cent of starting weight.

At autopsy there was liver discoloration and renal hyperemia macroscopically.

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TABLE III

SKIN IRRITATION IN RABBITS AFTER APPLICATION OF 'Pyranol 1470'

Sample Applied Undiluted

Animal Number	Numerical Evaluation At The End Of 1 Hour	24 Hours	48 Hours	72 Hours	120 Hours	168 Hours
1	3	5	4	3	2	1
2	2	4	3	2	1	0
3	3	5	4	2	2	1
Average	2.6	4.6	3.6	2.3	1.6	0.6

DISCUSSION -

The compound was classed as a moderate skin irritant.

The average maximum score was 4.6 out of a possible 8 in twenty-four hours.

Well-defined redness with very slight edema developed within an hour for an average score of 2.6 out of a possible 8. Overnight there was well-defined to moderate erythema and slight edema increasing the average score to 4.6. No blistering was observed. Inflammation gradually receded following removal of the application. After seven days one animal was given a score of zero but very slight redness was still present on the remaining animals.

TABLE IV

EYE IRRITATION IN RABBITS AFTER APPLICATION OF 'Pyranol 1470'

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	26	17	12	6	0
2	28	21	13	8	4	0
3	35	30	20	12	6	0
Average	32.6	25.6	16.6	10.6	5.3	0.0

DISCUSSION -

The compound was classed as a moderate eye irritant.

The average maximum score was 32.6 out of a possible 110 in one hour.

There was intermittent pawing at the eyes for several minutes following application.

After one hour the average score was 32.6 out of a possible 110. Slight edema, slight to moderate redness of the conjunctivae, copious discharge, and slight iris congestion developed. The corneal area cleared somewhat in twenty-four hours and within five days iris and cornea were normal. Conjunctivitis and edema cleared completely within seven days.

TABLE V

INHALATION OF 'Pyranol 1470' VAPORS BY RATS

Average Temperature Inside Chamber ..... 78° F.
Average Relative Humidity Inside Chamber ..... 42 %

Animal No. - Sex	Fate	Observations During Exposure
1 - Male	Survived	Occasional pawing at the eyes in ten to fifteen minutes ...
2 - Male	Survived	Slight nasal discharge ...
3 - Male	Survived	Some discomfort ...
4 - Male	Survived	No real weakness ...
		Occasional irregular breathing.

DISCUSSION -

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

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

The vapors produced mild nasal and eye inflammation as well as some discomfort. No weakness of consequence was noted at the end of exposure and no bronchial rales developed.

Activity and appetite returned to normal within twenty-four hours. Nasal inflammation disappeared within seventy-two hours. No respiratory complications developed during the ten day observation period.