YOUNGER LABORATORIES
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Certificate of Analysis

May 5th, 1969

SUBJECT -

Toxicological Investigation Of: A Functional Fluid Formulation
Monsanto Sample Number 52
Monsanto Project Number Y-69-42

STUDY CONDUCTED FOR -

Monsanto Company, St. Louis, Missouri

COPY

EXPERIMENTAL PROCEDURE -

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

The diluted 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 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 test animals were examined macroscopically.

The data, together with the dilution at which the compound was fed, 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 symptoms 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, Woodard and Calvery, et al, are shown in Table IV.

E) Vapor Inhalation (Male Rats)

Six mature male rats were placed in a metal chamber of 70 liters capacity and exposed for six hours to a concentrated atmosphere of vapors produced by passing a stream of air through 140.5 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 four 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, then sacrificed for macroscopic examination.

The data are shown in Table V.

SUMMARY -

A Functional Fluid Formulation

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

The Oral LD_{50} for male and female rats was placed at 1600 milligrams per kilogram with lower and upper limits of 1450 to 1760 milligrams per kilogram.

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

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, Mixed Sex)

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

The average maximum score was 2.3 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 6.0 out of a possible 110 in one hour.

E) Vapor Inhalation (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 practically non-toxic under conditions of the test.

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BY: MELVIN D. BIRCH

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TABLE I

THE ORAL LD_{50} OF

'A Functional Fluid Formulation' FOR RATS

Sample Fed As A 20.0% Solution In Corn Oil

Animal No. - Sex	Weight Gm.	Dose Mg./Kg.	Fate
1- Female	235	1260	Survived
2- Male	215	1260	Survived
3- Female	245	1260	Died
4- Male	260	1260	Survived
5- Female	245	1260	Survived
6- Male	220	1580	Survived
7- Female	225	1580	Died
8- Male	240	1580	Died
9- Female	230	1580	Died
10- Male	225	1580	Survived
11- Female	225	2000	Died
12- Male	240	2000	Died
13- Female	220	2000	Died
14- Male	240	2000	Died
15- Female	245	2000	Survived
16- Male	250	2510	Died
17- Female	245	2510	Died
18- Male	235	2510	Died
19- Female	230	2510	Died
20- Male	225	2510	Died

DISCUSSION -

The Oral LD_{50} for male and female rats was placed at 1600 milligrams per kilogram with lower and upper limits of 1450 to 1760 milligrams per kilogram.

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

Survival time was one to five days with most deaths occurring in two to three days.

Toxic symptoms included reduced appetite and activity for three to five days followed by increasing weakness, collapse, and death. Note: Most survivors showed slight weight loss at seven days.

At autopsy there was mottled and discolored liver, hemorrhagic lungs, gaseous and inflammed intestinal tract, and discoloration of the kidneys in some animals.

Surviving animals were sacrificed nine days after dosing. Macroscopic examination revealed discoloration of liver and kidneys (jaundice) and gastrointestinal inflammation in all dose levels.

TABLE II

THE MINIMUM LETHAL DOSE OF 'A Functional Fluid Formulation'
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	2.2	794	+ 0.1	Survived
2 - Male	2.1	1260	0.0	Survived
3 - Female	2.5	2000	- 0.3	Died -- 6 Days
4 - Male	2.0	3160	- 0.2	Died -- 7 Days
5 - Female	2.1	5010	- 0.5	Died -- 5 Days
6 - Male	2.3	7940	- 0.5	Died -- 5 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 seven days.

Toxic symptoms included reduced appetite and slight weakness in survivors; greatly reduced appetite and increasing weakness in animals that succumbed (#3 - #6).

At autopsy animals #5 and #6 showed marked yellowness of the liver, kidney discoloration, distended gall bladder, and distended vessels in intestinal tract. Animals #3 and #4 showed distended gall bladder and jaundiced liver.

Surviving animals were sacrificed fourteen days after dosing. Discoloration of the liver was observed; otherwise the viscera appeared normal by macroscopic examination.

TABLE III

SKIN IRRITATION IN RABBITS AFTER APPLICATION OF

'A Functional Fluid Formulation'

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 - Male	0	2	2	1	0	0
2 - Female	0	2	2	1	0	0
3 - Male	0	3	2	1	0	0
Average	0.0	2.3	2.0	1.0	0.0	0.0

DISCUSSION -

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

The average maximum score was 2.3 out of a possible 8 in twenty-four hours.

No skin changes were noted in one hour.

In twenty-four hours there was well-defined erythema in all three animals and barely perceptible edema on one animal.

Within forty-eight hours well-defined erythema remained; edema had disappeared.

In three days erythema reduced to slight.

Within five days all animals received a score of zero.

T A B L E IV

EYE IRRITATION IN RABBITS AFTER APPLICATION OF

'A Functional Fluid Formulation'

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 - Female	6	2	0	0	0	0
2 - Male	6	2	0	0	0	0
3 - Male	6	0	0	0	0	0
Average	6.0	1.3	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 6.0 out of a possible 110 in one hour.

Much discomfort with pawing, squealing, and eyes closed was observed immediately following application.

In ten minutes there was slight erythema, edema, and discharge, and the eyes remained closed.

In one hour there was no change. There was no corneal dullness.

Within twenty-four hours discharge had ceased, edema disappeared, and barely perceptible redness was noted on two animals. One animals received a score of zero.

Within forty-eight hours all animals received a score of zero.

TABLE V

INHALATION OF

'A Functional Fluid Formulation' VAPORS BY RATS

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

Average Relative Humidity Inside Chamber ..... 54 %

Amount of Sample -- To start ..... 140.5 Grams
Recovered ..... 139.0 Grams
Vaporized or left
in equipment ..... 1.5 Grams (1.0%)

Animal No. - Sex	Fate	Observations During Exposure
1 - Male	Survived	No immediate effect ...
2 - Male	Survived	No apparent discomfort or lethargy ...
3 - Male	Survived	
4 - Male	Survived	No weakness or nasal discharge ...
5 - Male	Survived	Normal respiration.
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.

There were no visible signs of weakness or distress of any consequence observed during the exposure period. Ocular and nasal mucosae remained free of inflammation.

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

Test animals were sacrificed ten days after exposure. Macroscopic examination revealed slight areas of lung congestion in all animals; otherwise the viscera appeared normal.