11-20

Scientific Associates

1474 S. Vandeventer Ave. • St. Louis 10, Mo.

January 13, 1954

11-30-53

CERTIFICATE OF ANALYSIS

SUBJECT -

(A) The Acute Oral Toxicity (LD_{50}) of Functional Fluid OS-54 Per Rate
(B) Inhalation of OS-54 Fumes By Rabbits Over Period of Three Days
(C) Ocular Irritation Produced by OS-54

TEST CONDUCTED FOR -

Monsanto Chemical Company, St. Louis, Missouri

Monsanto Project No.: SA-20

EXPERIMENTAL PROCEDURE -

(A) The LD_{50} was determined by the introduction of measured single doses of undiluted OS-54 into the stomachs of Sprague Dawley strain albino rats by means of a rubber catheter attached to a hypodermic syringe. Rats with a weight range of 175-245 grams were used in the test.

After the approximate Minimum Lethal Dose was found, groups of animals were fed at 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 Hilse (Quart. J. of Pharm. and Pharmacol. 11, 1938).

Selected animals from these receiving a lethal dose were autopsied and macroscopic examination made of the viscera. The data are found in Table I.

(B) The inhalation studies were conducted in a metal chamber approximately 3 feet long and 2 feet in diameter with a clear plastic door for observation. Four New Zealand white rabbits were placed inside the chamber and exposed to OS-54 that was atomized at room temperature by forcing compressed air through a container of the fluid. A great deal of frothing took place with only a light cloud of OS-54 particles appearing in the chamber. An air pressure of 10-12 pounds per square inch was used.

The rabbits were exposed to this atmosphere for four hours with no outward show of discomfort. Since the concentration of atomized OS-54 inside the chamber was only 0.34 milligram per liter, it was decided to place a chrome steel straight type heater inside the drum and drop the fluid from a burette onto a shallow copper dish attached to the heater which in turn was attached to a Powerstan variable transformer.

The OS-54 was volatilized at a temperature of 200°-200° C. with air intake at a rate of 1.0 liter per minute. Under these conditions a dense, whitish-gray fog was produced that obscured the animals excepting when they were near the transparent

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door. The rabbits were exposed daily for three consecutive days for a total exposure time of 20 hours. The survivors were held 7 days for observation after completion of the exposure. Six young mature albino rats were also placed in the drum alongside the rabbits the last day of exposure.

The concentration of vapors was found by passing a measured volume of chamber fumes through a train of three absorption flasks containing acetone and the optical density read on a Bausch Spectrophotometer at a wave length setting of 250 millimicrons. The acetone was first slowly evaporated off and the residue taken up in ethyl alcohol. The concentration per liter of air was found by comparison with a curve prepared from known amounts of CS-54 dissolved in ethyl alcohol. The data are given in Tables II and III.

(C) The degree of ocular irritation produced by CS-54 was found by using the technique developed by Draize, Woodard and Calvery (J. Pharmacology and Experimental Therapeutics, Vol. 82, No. 4, 1944). Briefly, it consisted of dropping 0.1 milliliter of the fluid into the conjunctival sac of the right eye of six albino rabbits and observing the effect over a period of several days. The left eye serves as a control. The scores are given in Table IV.

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DISCUSSION -

The LD_{50} of Functional Fluid OS-54 was calculated to be 13.50 grams per kilogram with lower and upper limits of 12.69-14.43 grams per kilogram. Survival time for those animals receiving a lethal dose varied from 30 hours to 8 days. There was moderate to severe diarrhea in all animals dosed at the levels shown in the table. Survivors lost part of the hair on the underside of their bodies. However, the hair grew back after several weeks.

At autopsy of animals receiving a lethal dose, the intestinal tract was gaseous and irritated, and both the liver and spleen were abnormally dark.

TABLE II

FATE AND WEIGHT CHANGES OF RABBITS EXPOSED TO OS-54 VAPORS

Animal No.- Sex	Initial Weight Kilograms	Weight Change 7 Days After Exposure Expressed As % of Initial Weight	Fate
1- Male	2.0	---	Died--2nd Day
2- Male	1.8	0.0	Survived
3- Male	2.1	---	Died--2nd Day
4- Male	2.2	---	Died--3rd Day

TABLE III

AVERAGE DAILY CONCENTRATION OF OS-54 VAPORS IN CHAMBER

Day	Concentration Of OS-54 Vapors Mg./Liter	Average Temperature Inside Chamber °F.	Average Relative Humidity Inside Chamber %
First	2.80	84.0	62.0
Second	2.53	82.5	56.8
Third	2.71	87.3	60.5
Average	2.69	84.6	59.8

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Scientific Associates Certificate of Analysis — Page 3 (1-13-54)

TABLE I

THE ACUTE ORAL TOXICITY (LD_{50}) OF
FUNCTIONAL FLUID OS-34 FOR RATS

Animal No.- Sex	Weight Grams	Dose G./Kg.	Fate
1- Male	235	9.07	Survived
2- Male	220		Died
3- Female	190		Survived
4- Female	175		Survived
5- Male	240		Survived
6- Male	230		Died
7- Male	245		Survived
8- Female	200		Survived
9- Female	185		Survived
10- Female	205		Survived
11- Male	220		Survived
12- Male	240	12.10	Died
13- Male	210		Died
14- Male	225		Survived
15- Female	180		Survived
16- Female	195		Survived
17- Female	175		Died
18- Male	220		Survived
19- Male	240		Survived
20- Female	200		Survived
21- Female	190		Died
22- Female	230		Survived
23- Female	210		Survived
24- Male	245	15.12	Died
25- Male	225		Died
26- Male	230		Died
27- Female	185		Survived
28- Female	205		Died
29- Male	230		Survived
30- Male	215		Died
31- Male	235		Died
32- Male	240		Died
33- Male	240		Survived
34- Female	180		Died
35- Female	200	18.15	Died
36- Female	185		Died
37- Female	195		Survived
38- Male	225		Survived
39- Male	220		Died
40- Male	210		Died
41- Male	235		Survived
42- Male	235		Died
43- Female	200		Died
44- Female	185		Died

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Scientific Associates Certificate of Analysis — Page 5 (1-11-54)

DISCUSSION -

The four rabbits survived the first day's exposure although they were rather exhausted when taken out of the chamber. The fumes produced moderately severe nasal irritation that caused the animals to *search* every corner of the drum in an effort to escape them. During the second day when two of the animals died, a nasal discharge, consisting of tales yellowish mucus, appeared. At the end of the second day, bronchial rales were evident in the two survivors. Both recovered reasonably well overnight but one died during the third day's exposure. The survivor regained its normal appetite within a few days and at the end of the 7 days observation period appeared to be in satisfactory condition.

The fumes caused moderate ocular erythema with mild edema. Lacrimation was moderate but cleared up soon after the animals were taken out of the drum each day.

The six rats exposed for approximately seven hours to the vapors survived without incident although the degree of nasal and ocular irritation was comparable to that for the rabbits.

The average concentration of CS-54 fumes was 2.69 milligrams per liter of air at an average temperature of 84.6° F. and relative humidity of 59.8%. Approximately 30% of the fluid was volatilized under the conditions of the test.

Macroscopic examination at autopsy of rabbits dying in the chamber revealed severe pulmonary congestion with numerous hemorrhagic areas present.

TABLE IV

SCORES REPRESENTING THE SEVERITY OF
OCULAR IRRITATION PRODUCED BY
FUNCTIONAL FLUID CS-54

	NUMERICAL EVALUATION AT THE END OF				
Animal No.	1 Hour	4 Hours	24 Hours	48 Hours	72 Hours
1	24	26	18	8	0
2	18	22	16	8	0
3	28	24	18	10	0
4	22	22	16	10	0
5	24	28	22	10	0
6	20	20	12	6	0
Average	22.7	23.7	17.0	8.6	0.0

DISCUSSION -

Ocular irritation consisted of moderate erythema with slight to moderate edema developing within the first hour for an average score of 22.7 out of a possible score of 110. The iris and cornea were only slightly affected although they were somewhat dull for several hours because of the coating of fluid. Edema was confined principally to the eyelids. The count had lowered to 17.0 after 24 hours and all signs of irritation had disappeared within 72 hours.

SUMMARY -

(A) The Acute Oral Toxicity of Functional Fluid CS-5A for rats was found to be 13.50 grams per kilogram with fiducial limits of 12.59-14.43 grams per kilogram.

(B) Four rabbits survived one day's exposure to CS-5A fumes at a concentration of 2.60 milligrams per liter of air. Two of the animals died during the second day's exposure while a third died during the third day. The fumes were rather dense in the chamber.

A group of rats exposed for one day under the same conditions survived without incident.

It was concluded that CS-5A fumes are reasonably toxic in heavy concentrations over an extended period of exposure.

(C) CS-5A was mild to moderately irritating to the eyes of rabbits. An average maximum score of 21.7 out of a possible 110 was recorded 4 hours after the fluid was placed in the eyes.

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