YOUNGER LABORATORIES
Biochemists . . . Pharmacologists . . . Analysts

ROUTE 9 — BOX 281
ST. LOUIS 23, MISSOURI
PHONE: TILDEN 6-2540

Certificate of Analysis

February 22nd, 1958

SUBJECT -

Toxicological Investigation Of 'OS-95.'

Monsanto Sample Number 17
Monsanto Project Number X-58-4

TEST CONDUCTED FOR -

Monsanto Chemical Company, St. Louis, Missouri

EXPERIMENTAL PROCEDURE -

A) Oral LD_{50} In 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 rats 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 Bliss.

The viscera of the animals that succumbed was examined macroscopically.

The data are shown in Table I.

B) Skin Absorption With Rabbits
The sample was applied undiluted in varying amounts to the closely clipped, intact skin of New Zealand white rabbits and the treated areas covered with plastic shields to prevent evaporation.

Observations were made for toxic symptoms and the viscera of those animals that succumbed was examined macroscopically.

The data are found in Table II.

C) Skin Irritation In Rabbits
OS-95 was applied undiluted to the clipped, intact skin of three albino rabbits and removed after twenty-four hours with soap and water.

Observations were made over a period of several days for inflammation.

Irritation was scored according to the method of Draize, Woodard and Calvery (Journal of Pharm. and Exp. Therapeutics, Vol. 82, No. 4, Dec. 1944).

The results are given in Table III.

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EXPERIMENTAL PROCEDURE - (Continued)

D) Eye Irritation In Rabbits
0.1 Milliliter of sample was placed in the conjunctival sac of the right eye of three albino rabbits. The eyes were rinsed with warm isotonic saline solution after twenty-four hours.

Observations were made over a period of several days for irritation.

The data, scored according to Draize et al, are found in Table IV.

E) Vapor Inhalation In Rats
Four mature male rats were placed in a metal chamber of approximately 25 liters capacity and exposed for six hours to a concentrated atmosphere of CS-95 vapors produced by aspirating the liquid at room temperature.
The sample (250 grams) was poured into a three hole, round bottom flask and compressed air passed over an orifice, the lower end of which was immersed in the liquid. A rubber tube led from the flask into the chamber. A glass impinger was placed in the line to break up droplets and allow, as nearly as practicable, only vapors to pass.
The animals were checked for behavior and, since there were no deaths, all were held for ten days observation.
The data are shown in Table V.

SUMMARY -

The Oral LD_{50} of CS-95 was found to be 10,500 milligrams per kilogram with lower and upper limits of 9,295 to 11,865 milligrams per kilogram.

The Minimum Lethal Dose range by Skin Absorption in rabbits was 625 to 1,250 milligrams per kilogram.

CS-95 was classed as a moderate skin irritant.

CS-95 was classed as a mild eye irritant.

No deaths resulted when rats were exposed for six hours to a concentrated atmosphere of CS-95 vapors.

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Fred M. Younger

BY: FRED M. YOUNGER

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— Monsanto Chemical Company

TABLE I

THE ORAL LD_{50} OF 'CS-95'
FOR RATS

Animal No. - Sex	Weight Gm.	Dose Mg./Kg.	Fate
1- Male	270	9,000	Survived
2- Female	195	9,000	Survived
3- Female	210	9,000	Survived
4- Female	190	9,000	Survived
5- Male	230	9,000	Survived
6- Female	205	10,000	Died
7- Female	195	10,000	Survived
8- Male	215	10,000	Survived
9- Male	230	10,000	Died
10- Male	245	10,000	Survived
11- Female	185	11,000	Died
12- Female	190	11,000	Died
13- Female	210	11,000	Survived
14- Female	225	11,000	Died
15- Male	240	11,000	Survived
16- Male	220	12,500	Died
17- Female	215	12,500	Died
18- Female	200	12,500	Died
19- Female	190	12,500	Survived
20- Male	195	12,500	Died

DISCUSSION -

The Oral LD_{50} was placed at 10,500 milligrams per kilogram with lower and upper limits of 9,295 to 11,865 milligrams per kilogram.

Survival time was overnight to three days.

There was diarrhea, salivation, tremors, and coma.

At autopsy the gastrointestinal tract was gaseous and inflamed. There was liver discoloration due in part to congestion.

TABLE II

THE MINIMUM LETHAL DOSE OF 'OS-95' BY SKIN ABSORPTION IN RABBITS

Animal No. - Sex	Weight Kg.	Dose Mg./Kg.	Weight Change 5 Days After Dosing Kg.	Fate
1 - Female	2.3	450	- 0.2	Survived
2 - Female	2.1	625	- 0.2	Survived
3 - Male	2.6	1,250	- 0.3	Died - 11 Days
4 - Male	2.6	2,500	- 0.2	Died - 5 Days
5 - Male	2.4	5,000	- 0.2	Died - 5 Days
6 - Female	2.1	7,500	- 0.4	Died - 4 Days
7 - Male	2.5	10,000	- 0.4	Died - 3 Days

DISCUSSION -

The Minimum Lethal Dose range was found to be 625 to 1,250 milligrams per kilogram.

Survival time was three to eleven days.

Appetite was soon affected resulting in weight losses. This was about the only toxic symptom for two days. Weakness and lethargy were noted thereafter. Violent convulsions developed in two of the animals several hours before death.

At autopsy there were extensive hemorrhagic areas in the lungs and moderate kidney congestion.

TABLE III

SKIN IRRITATION IN RABBITS AFTER APPLICATION OF
'OS-95'

Animal Number	Numerical Evaluation At The End Of 2 Hours	24 Hours	48 Hours	72 Hours	120 Hours
1	2	3	2	1	1
2	2	3	2	2	1
3	1	3	2	1	1
Average	1.6	3.0	2.0	1.3	1.0

DISCUSSION -

After two hours there was slight to well defined erythema with no edema for an average score of 1.6 out of a possible 8.0. Overnight the average score increased to 3.0 on the basis of more redness and slight edema.

The application was removed in twenty-four hours but the irritation decreased rather slowly. Slight erythema was still present on all animals on the fifth day.

OS-95 was classed as a moderate skin irritant.

TABLE IV

EYE IRRITATION IN RABBITS AFTER APPLICATION OF
'OS-95'

Animal Number	Numerical Evaluation At The End Of 1 Hour	24 Hours	48 Hours	72 Hours	120 Hours
1	12	8	4	2	0
2	10	6	4	0	0
3	12	8	4	2	0
Average	11.3	7.3	4.0	1.3	0.0

DISCUSSION -

There was mild immediate discomfort shown upon application. The average score after one hour was 11.3 out of a possible 110.0. Irritation consisted of moderate erythema of the conjunctivae with mild edema and lacrimation. The details of the iris were slightly obscured due to some congestion. The latter condition improved overnight resulting in an average score of 7.3. One animal was free of inflammation in seventy-two hours and all were given a score of zero in five days.

OS-95 was classed as a mild eye irritant.

TABLE V

INHALATION OF 'CS-95' VAPORS BY RATS

Average Temperature Inside Chamber . . . . . 76.0° F.
Average Relative Humidity Inside Chamber . . . . . 59.5 %
Amount Of 'CS-95' Vaporized. . . . . 6.4 Grams

Animal No. - Sex	Fate	Observations During Exposure
1 - Male	Survived	Little immediate reaction -
2 - Male	Survived	Activity about normal -
3 - Male	Survived	Animals continue to explore chamber -
4 - Male	Survived	Discomfort slight after four hours.

DISCUSSION -

The rats survived the six hour exposure with no aftermath of bronchial rales or other evidence of respiratory injury. Activity was not affected to any noticeable extent. The animals remained steady and were observed eating and drinking at intervals. Nasal and ocular irritation was not sufficient to stimulate a discharge.

6.4 Grams, equivalent to 2.5 per cent of the sample, was vaporized. Considerable agitation was maintained at a pressure of three pounds per square inch. However, the chamber atmosphere remained quite clear with only a very slight haze detectable by reflected light.

It was concluded that a concentrated atmosphere of CS-95 vapors was only slightly irritating to rats exposed for six hours.