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Major, C. W. S.

E.A.T.R. 119, COPY NO. 1.

# TOXICITY OF PHOSGENE TO WHITE MICE BY INHALATION.

By

W.J.H.B. Wells, A.B., Ph.G.

AD-B956 567

WAR DEPARTMENT
CHEMICAL WARFARE SERVICE
EDGEWOOD ARSENAL, MD.

COLONEL C. E. BRIGHAM, COMMANDING

MAJOR E. MONTGOMERY, TECHNICAL DIRECTOR

COLONEL E. B. VEDDER, CHIEF, MEDICAL RESEARCH DIV.

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July 11, 1939.

Correction of Report E.A.T.R. 119.

Through: Technical Director.

To: Chief, Information Division, Edgewood Arsenal, Md.

1. At the top of pages 6 and 7 of E.A.T.R. 119 the title of the column now reading "concn. of phosgene" should read "Concn. of chlorine". This error was made when the final copies of this report were typed.

B. H. ADAMS,
Commander (MC), USN
Act'g Chief, Medical Research Division.

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# TOXICITY OF PHOSGENE TO WHITE MICE BY INHALATION.

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**By**

**W.J.H.B. Wells, A.B., Ph.G.**


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# TOXICITY OF PHOSGENE TO WHITE MICE BY INHALATION.

## ABSTRACT


**Object.**

The object of this work was to provide accurate and adequate data on the toxicity of phosgene by inhalation on white mice for a 10-min. exposure.

**Discussion.**

In the case of a gas like phosgene, where the chief cause of death is pulmonary edema, it is possible that deaths occurring within 48 hr. after exposure are the best index of the toxicity of the gas. However, in order to provide all figures that may be required for comparison with other gases 5-da. and 10-da. mortalities are also given.

**Conclusions.**

The experimental lethal concentration for phosgene for a 10-min. exposure, and an observation period of 2 da. (48 hr.), can be placed at 0.375-0.377 mg./l.

The experimental lethal concentration for phosgene for a 10-min. exposure, and an observation period of 5 da., is slightly greater than 0.366 mg./l.

The median lethal concentration for phosgene, as read from the curve, for a 10-min. exposure (48 hr. observation), is 0.375 mg./l.


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# TOXICITY OF PHOSGENE TO WHITE MICE BY INHALATION.

## I. INTRODUCTION.

The object of this work was to provide accurate data on the toxicity of phosgene to white mice. The work was carried out under Project A 10.2 as part of the general program authorized for the Fiscal Year 1932. The new data constitute a revision of the data on this standard agent included in the revised summary of mouse toxicity data now being prepared (E.A.T.R. 109) under Project A 10 and are also of general interest in connection with Project A 3 for the development of a nonpersistent agent superior to phosgene.

## II. HISTORICAL.

Phosgene was known and used during the World War; consequently, much toxicity work was done on it both in Europe and the United States. One European source (Ph 1 CXVIII) shows a series of concentrations and periods of exposure, but fails to establish a lethal figure. Miller and Gross (B.M. XXXII, 57) established 0.075 mg./l. as the lethal concentration for mice by inhalation for a 30-min. exposure and a 10-da. observation period. These same investigators placed 0.008 mg./l. as the lethal concentration for a 4-hr. exposure, but this figure can be questioned since insufficient work was done to really establish a lethal concentration. Kuhn and Cohn (P.T. II, A-127), on very meager data, places 0.4 mg./l. as the lethal concentration for white mice by inhalation for a 10-min. exposure.

## III. THEORETICAL.

E.A.T. Report 62, which describes preliminary toxicity and skin-irritant test methods used at Edgewood Arsenal, defines the lethal concentration as "that which kills 50% or more of the exposed mice" and states that this concentration will be known in future as the "minimum lethal concentration (M.L.C.) for mice, 10 minutes". J.W. Trevan, in an article entitled "The error of determination of toxicity", Proc. Roy. Soc. Ser. B, 101, 483-514, 1927), calls attention to the lack of accurate definition of such terms as "minimal lethal dose" which may mean the dose just sufficient to kill only an occasional animal, or that which kills 50% or that which is just large enough to kill all the animals. It seems desirable to clear up this looseness of definition and we have accordingly adopted the expression "median lethal

concentration" suggested by Trevan and define it as the concentration which will kill exactly 50% of a large group of animals exposed to it. It will be determined by plotting the data on concentration vs. per cent mortality coordinates, drawing a smooth S-shaped probability curve through the points and reading from this curve the concentration which produces 50% deaths. For the purpose of comparison with earlier methods used at this Arsenal, estimates will also be made based on runs which gave exactly 50% deaths, or thereabouts, and these estimates will be termed the "experimental lethal concentration".

In order to eliminate as far as is practicable the error involved in testing small groups of animals, groups of not less than 10 mice will be tested at a time. A new apparatus large enough to allow 20 mice to be used in one run has been built and is described for the first time in this report.

## IV. EXPERIMENTAL.

### A. Material.

The phosgene used was produced at Edgewood Arsenal and had the following purity:

Phosgene ($COCl_2$), 99.9% based on total chlorine.
Free chlorine, none.

White mice were used as subjects in this work.

### B. Apparatus.

This new apparatus was built up around an air-tight metal chamber which in the future shall be known as "metal chamber for instantaneous exposure of mice". This chamber is made of steel boiler plate about 1/4 in. thick and welded in all seams. The chamber has the following dimensions: 35.9 in. long, 35.9 in. wide, and 29.8 in. high, and has a capacity of 22.23 cu.ft. or 629.4 liters.

On the front the chamber is equipped with a large circular opening directly in the center and two small openings in opposite corners. The large opening is closed and made air-tight with a large disc of steel plate equipped with a rubber gasket and

held in position by twelve screw clamps. One of the small openings is closed and made air-tight by a small disc of steel plate equipped with a gasket and held in place by "hex" cap screws. The other opening serves as the entrance for the gas-air mixture. The top of the chamber is solid and not equipped with any openings. The back of the chamber is equipped with two small openings similar to those found on the front. These openings on the back are also closed and made air-tight by small discs of steel plate fitted with rubber gaskets and held in place by "hex" cap screws.

The two sides of the chamber are fitted with wired-glass gas-tight windows, and these sides are also pierced by small openings which are closed and made air-tight by small discs of steel plate fitted with rubber gaskets and held in place by "hex" cap screws. On the left side of the chamber one of the smallopenings has been fitted with a cylindrical container, of perforated metal, (see Photo. 6135) for holding the mice to be gassed. This container has a hinged top to facilitate the introduction and removal of mice. This container is also fitted at its ends with steel discs in order to make the opening air-tight both when the container is outside and inside the chamber.

The bottom of this chamber is solid save for a one-inch pipe which is exactly in the center. This pipe is fitted with a screw cap at its lower end, and its upper end is flush with the inside surface of the floor of the chamber. The pipe was installed to facilitate cleaning the chamber and as an opening through which samples could be drawn.

The most important feature of the entire apparatus is the air suction control. This control apparatus (see Photo. 6136) is composed of: (1) a plate of stainless steel having a 3/4-in. knife-edge orifice (other orifice plates having openings of 1/2 in. and 7/8 in. are also provided), (2) a water manometer to measure the resistance on either side of the orifice plate, (3) a valve to control the air flow, (4) an equalising tank. This part of the apparatus was developed by the Plants Department of the Munitions Development Division, who rendered invaluable services in its planning and installation. Calculated calibration curves for the orifice plates are shown on the attached blueprint.

C. Procedure.

1. Operation of Apparatus.

The sample of phosgene used in this work was in a

cylinder (1), (Photo. 6135). It was passed over an equalizer filled with liquid petrolatum then through a flowmeter (2) to measure the flow. From flowmeter (2) it passed into the mixing bowl (4) where it was mixed with air from the room, and from the mixing bowl (4) it passed into the metal chamber (5) The wet and dry bulb thermometers (3) were placed 2 in. from the outside opening of the mixing bowl (4). The mouse container (6) was used to expose the animals instantaneously to a desired concentration of phosgene by pushing it into the chamber (5). The wet meter (7) was used to measure the sample drawn from the chamber (5) through the sampling bottles (8). (For further details see Photo. 6135.)

The gas-air mixture was drawn from the chamber (5) (Photo. 6136) by a pump operated by an electric motor (4) through a 3/4-in. knife-edge orifice cut in a stainless steel plate (1) which was equipped with a water manometer (2) to measure the flow through the chamber in inches, thence through a metal equalizing chamber (5). After passing through the pump (4) the gas-air mixture was passed on into the exhaust line of the hood in the building and thus to the outside air. In the line between the orifice plate (1) and the equalizing tank (5) was a valve (3) to regulate the flow through the chamber. The electric motor (6) and the pump (6') were used to draw gas-air samples from the chamber. (For further details of the air suction control apparatus see Photo. 6136.)

2. Distribution of Gas-Air Mixture in Metal Chamber.

The following checks were made for the purpose of showing that the concentration in the chamber was uniform throughout. Such tests have been made before in similar gassing chambers but were not recorded in reports. Chlorine was used on account of its comparative simplicity of analysis in gas-air mixtures and also for the accuracy of the analysis.

The same air flow through the chamber (3/4-in. orifice plate) was used for this test as was used in the phosgene and cyanogen chloride toxicity experiments, namely, 500 l. of air/min. (5.5 in. of water on the manometer). The flow of chlorine over a water manometer into the mixing bowl of the chamber through which 500 l. of air/min. was drawn had a differential ranging from 34.8 cm. to 35.1 cm. for all runs. All gas-air samples for analysis were drawn with aspirating bottles at the rate of one liter a minute for 3 min. The gas-air mixtures were collected in petticoat bubblers each containing 50 cc. of a 2% aqueous solution of potassium iodide. The sample thus collected

was titrated directly in the bubbler with N/100 sodium thiosulfate solution (0.009916), using 1 cc. of starch as the indicator.

Nine sampling points were used for collecting the samples inside the chamber. Check runs were made on each sampling position and the eight corner positions of the chamber were checked by drawing simultaneous samples against No. 1 sampling position.

The sampling positions were as follows:

Position 1: The center of the chamber.

Position 2: Four inches over the mouse container.

Position 3: Eight inches from the corner of the chamber at the suction outlet, (orifice plate).

Position 4: Eight inches from the corner of the chamber directly diagonal from the mouse container (lower level).

Position 5: Eight inches from the corner of the chamber directly diagonal from the suction outlet (lower level).

Position 6: Eight inches from the corner of the chamber directly over the mouse container.

Position 7: Eight inches from the corner of the chamber over the suction outlet.

Position 8: Eight inches from the corner of the chamber directly over number 4 position.

Position 9: Eight inches from the corner of the chamber directly in front of the mixing bowl.

In this manner all eight corners of the chamber were checked against the center.

| | | Titer | Conon. of ~~phosgene~~ $Cl_2$ | A.T.* | W.B.* | Flowmeter differential |
|---|---|---|---|---|---|---|
| | | cc. | mg./l. | °F. | °F. | cm. |
| Run 1 | Position 1 | | | | | |
| | 1st bubbler | 8.3 | 0.973 | 78 | 59 | 34.8 |
| | 2nd bubbler | 0.0 | 0.0 | | | |
| | Position 2 | | | | | |
| | 1st bubbler | 8.2 | 0.961 | | | |
| | 2nd bubbler | 0.0 | 0.0 | | | |
| Run 2 | Position 1 | | | | | |
| | 1st bubbler | 8.05 | 0.943 | 78 | 59 | 34.8 |
| | 2nd bubbler | 0.0 | 0.0 | | | |
| | Position 2 | | | | | |
| | 1st bubbler | 8.0 | 0.938 | | | |
| | 2nd bubbler | 0.0 | 0.0 | | | |
| Run 3 | Position 1 | | | | | |
| | 1st bubbler | 8.3 | 0.973 | 79 | 59 | 35.1 |
| | 2nd bubbler | 0.0 | 0.0 | | | |
| | Position 6 | | | | | |
| | 1st bubbler | 8.3 | 0.973 | | | |
| | 2nd bubbler | 0.0 | 0.0 | | | |

**Note:** The use of the second bubbler was discontinued after obtaining the above three sets of blanks.

| | | | | | | |
|---|---|---|---|---|---|---|
| Run 4 | Position 1 | 8.4 | 0.984 | 79 | 60 | 35.1 |
| | Position 6 | 8.4 | 0.984 | | | |
| Run 5 | Position 1 | 8.3 | 0.973 | 79 | 60 | 35.1 |
| | Position 5 | 8.3 | 0.973 | | | |
| Run 6 | Position 1 | 8.35 | 0.979 | 79 | 60 | 35.1 |
| | Position 5 | 8.35 | 0.979 | | | |
| Run 7 | Position 1 | 8.25 | 0.967 | 79 | 60 | 35.1 |
| | Position 9 | 8.15 | 0.955 | | | |
| Run 8 | Position 1 | 8.3 | 0.973 | 79 | 60 | 35.1 |
| | Position 9 | 8.3 | 0.973 | | | |
| Run 9 | Position 1 | 8.25 | 0.967 | 81 | 61 | 35.1 |
| | Position 3 | 8.35 | 0.979 | | | |
| Run 10 | Position 1 | 8.4 | 0.984 | 81 | 61 | 35.1 |
| | Position 3 | 8.3 | 0.973 | | | |
| Run 11 | Position 1 | 8.3 | 0.973 | 81 | 60 | 35.1 |
| | Position 4 | 8.3 | 0.973 | | | |
| Run 12 | Position 1 | 8.4 | 0.984 | 80 | 60 | 35.1 |
| | Position 4 | 8.3 | 0.973 | | | |
| Run 13 | Position 1 | 8.3 | 0.973 | 82 | 61 | 35.1 |
| | Position 8 | 8.3 | 0.973 | | | |

| | | Titer | Concn. of phosgene | A.T.* | W.B.* | Flowmeter differential |
|---|---|---|---|---|---|---|
| | | cc. | mg./l. | °F. | °F. | cm. |
| Run 14 | Position 1 | 8.3 | 0.973 | 82 | 61 | 35.1 |
| | Position 8 | 8.3 | 0.973 | | | |
| Run 15 | Position 1 | 8.3 | 0.973 | 83 | 61 | 35.1 |
| | Position 7 | 8.3 | 0.973 | | | |
| Run 16 | Position 1 | 8.3 | 0.973 | 81 | 60 | 35.1 |
| | Position 7 | 8.3 | 0.973 | | | |

*A.T. = air temperature.
W.B. = wet-bulb temperature.

The above data indicate that the distribution of the gas-air mixture is uniform throughout the chamber.

3. Determination of Concentrations of Phosgene.

The following technique was employed in determining the concentrations of phosgene used in the tests. A 4% solution of sodium peroxide, which had been boiled to remove the oxygen was used to absorb the phosgene. A pair of petticoat bubblers, each containing 50 cc. of this solution, was used for each exposure. Thirty liters of the gas-air mixture, measured by a wet meter, were drawn through the bubblers by a pump, at such a rate that the sample was being continuously withdrawn during the 10-min. run.

The contents of the bubblers were then washed into a beaker, care being taken to keep the volume below 300 cc.; 10 cc. nitric acid (conc.), and 20 cc. N/50 silver nitrate were added. The precipitated silver chloride was removed by filtrations and the excess $AgNO_3$ back-titrated with N/100 potassium thiocyanate using 1 cc. of ferric alum as an indicator. Calculations were made according to the formula:

$$\frac{((\text{cc. } AgNO_3 \times \text{N.F.}) - (\text{cc. KSCN} \times \text{N.F.})) \times 1/2 \text{ mol. wt. phosgene}}{1000} \times \frac{1000}{\text{Vol. sample in liters}} = \text{mg. phosgene per liter.}$$

Experimental runs using a third bubbler, upon analysis, showed no phosgene carried through the second bubbler. Therefore it was only necessary to use two bubblers in this work. All phosgene samples were taken from position (1).

4. Subjects.

White mice weighing from 18-30 g. were used for the toxicological study of phosgene. One hundred white mice were set aside in order to determine the normal death rate for 30 da. The normal death rate on one hundred white mice for 30 da. was found to be 44. This gives a daily death rate of 1.46%.

After the desired concentration of phosgene had been obtained in the chamber (5) as shown by a check run, the white mice were exposed to the gas instantaneously by placing them in the mouse container (6), (Photo. 6135), and then pushing the container into the chamber. Exposure was for 10 min.

D. Results.

The data on all the runs made on phosgene follow:

Table 1.

Exposure of Mice to Phosgene.

| Run No. | Date | Concen. | Air temp. | Wet bulb | Rel. hum. | No. mice used | No. mice dead 2 da. | 5 da. | 10 da. | Diff. on flow-meter | Time to take gas-air samples | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1932 | mg./l. | °F. | °F. | % | | | | | cm. | min. | sec. |
| *1 | 11/8 | 0.366 | 68 | 63 | 76 | 10 | 3 | 5 | 6 | 30 | 8 | 58 |
| 2 | 11/8 | 0.377 | 69 | 62 | 67 | 10 | 5 | 7 | 8 | 30 | 9 | 2 |
| 3 | 11/8 | 0.366 | 68.5 | 63 | 71.5 | 10 | 3 | 3 | 3 | 30 | 8 | 56 |
| 4 | 11/8 | 0.337 | 73 | 69 | 72 | 10 | 2 | 2 | 2 | 30 | 8 | 47 |
| 5 | 11/9 | 0.375 | 74 | 62 | 50 | 10 | 5 | 5 | 9 | 39 | 8 | 6 |
| **6 | 11/10 | 0.409 | 70 | 59 | 51 | 19 | 15 | 17 | 17 | 39.8 | 8 | 19 |
| 7 | 11/10 | 0.396 | 72 | 59 | 45 | 20 | 17 | 18 | 18 | 39.6 | 8 | 9 |
| 8 | 11/11 | 0.456 | 76 | 63 | 48 | 20 | 19 | 20 | 20 | 44.5 | 8 | 17 |

*No. 1 capillary used in tests 1 to 5, incl.
**No. 2 capillary used in tests 6 to 8, incl.

In all runs tabulated above the length of exposure of the mice to phosgene was 10 min., 30 l. of the gas-air mixture was drawn for analysis, and the air flow through the chamber was 500 l./min.

Capillary No. 1 was 4.1 cm. long and had a 1/2-mm. bore.

Capillary No. 2 was 8.0 cm. long and had a 1/4-mm. bore.

Pertinent toxicity data from Table 1 are rearranged in Table 2.

Table 2.

Toxicity of Phosgene to Mice.

| Concn. | Number of mice used | No. of mice dead 2 da. | 5 da. | 10 da. | Total deaths |
|---|---|---|---|---|---|
| mg./l. | | | | | |
| 0.456 | 20 | 19 | 20 | 20 | 20 |
| 0.409 | 19 | 15 | 17 | 17 | 17 |
| 0.396 | 20 | 17 | 18 | 18 | 18 |
| 0.377 | 10 | 5 | 7 | 8 | 8 |
| 0.375 | 10 | 5 | 5 | 9 | 9 |
| 0.366 | 10 | 3 | 5 | 6 | 6 |
| 0.366 | 10 | 3 | 3 | 3 | 3 |
| 0.337 | 10 | 2 | 2 | 2 | 2 |

## V. DISCUSSION.

In the case of a gas like phosgene, where the chief cause of death is pulmonary edema, it is possible that the 48-hr. deaths are the best index of the toxicity of the gas. However, in order to provide all figures that may be required for comparison with other gases, 5-da. and 10-da. mortalities are also given in the table. The 2-da. mortality figures have been used in the accompanying plot from which the median lethal concentration can be read.

## VI. CONCLUSIONS.

The experimental lethal concentration for 50% deaths for phosgene for a 10-min. exposure, and an observation period of 2 da. (48 hr.), may be placed at 0.375-0.377 mg./l., (see Table 2).

The experimental lethal concentration for 50% deaths for phosgene for a 10-min. exposure, and an observation period of 5 da., is slightly greater than 0.366 mg./l., (see Table 2).

The median lethal concentration for phosgene, as read from the curve, for a 10-min. exposure, and an observation period of 48 hr., is 0.375 mg./l.

Submitted:

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Work started: Nov. 8, 1932.
Work completed: Nov. 21, 1932.

Toxicity of Phosgene to White Mice by Inhalation.

E.A.T.R. 119.

Project A 10.2.

8 copies made)
Typed - mco)

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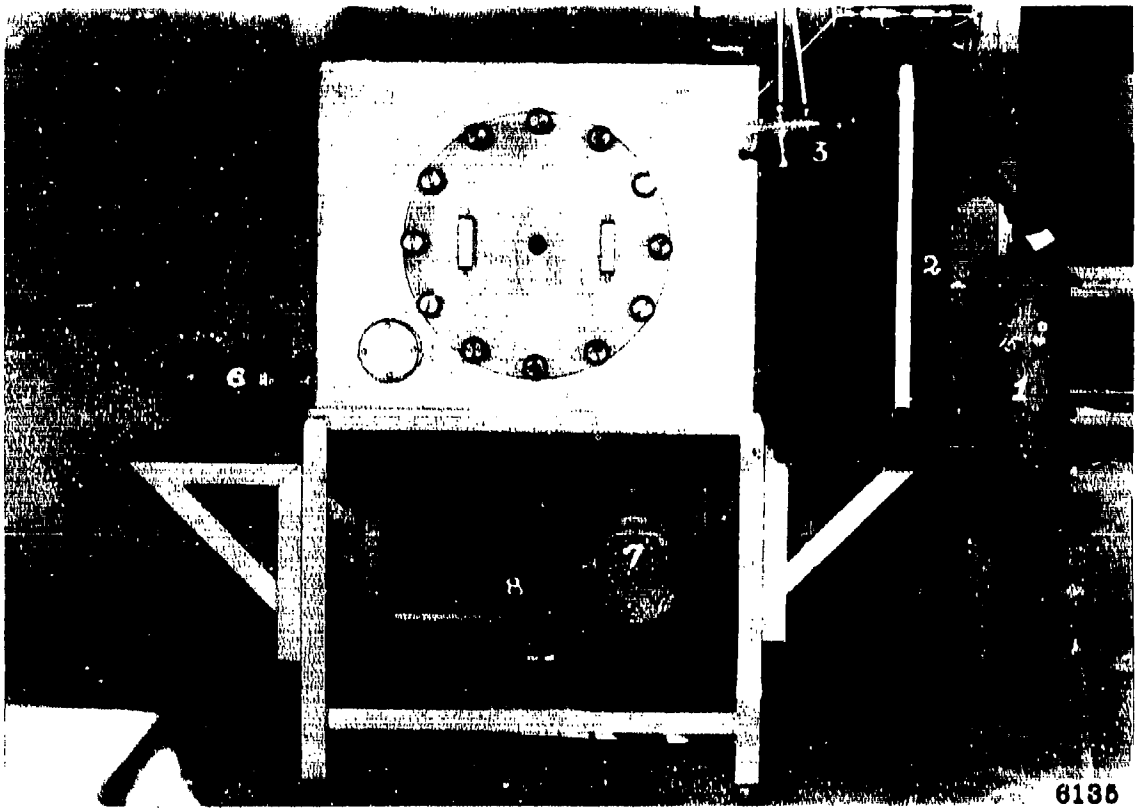


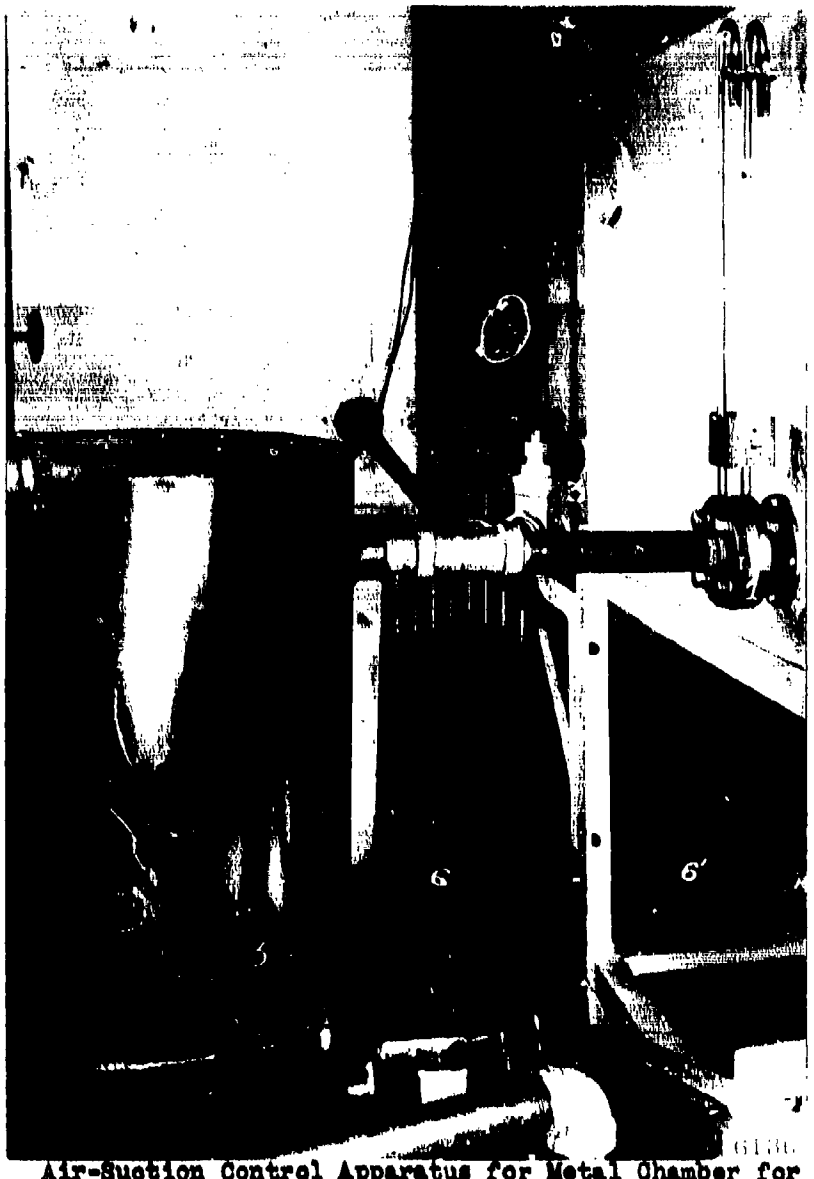

**Metal Chamber for Instantaneous Exposure of Mice.**

**OBJECT:** To show set up used in gassing mice in gas-air mixtures of cyanogen chloride and gas-air mixtures of phosgene.

**DESCRIPTION:** (1) Gas cylinder. (2) Flowmeter for measuring inflowing gas. (3) Wet and dry bulb recorders for incoming air. (4) Mixing bowl. (5) Chamber. (6) Mouse container, which slides into chamber. (7) Wet meter for measuring liters of air from mixture drawn through gas sampling bottles. (8) Gas sampling bottles.

**PROJECT:** A 10.2.

**DATE:** Photographed at Edgewood Arsenal, Md., Nov. 28, 1932, for E.A.T.R. 119.



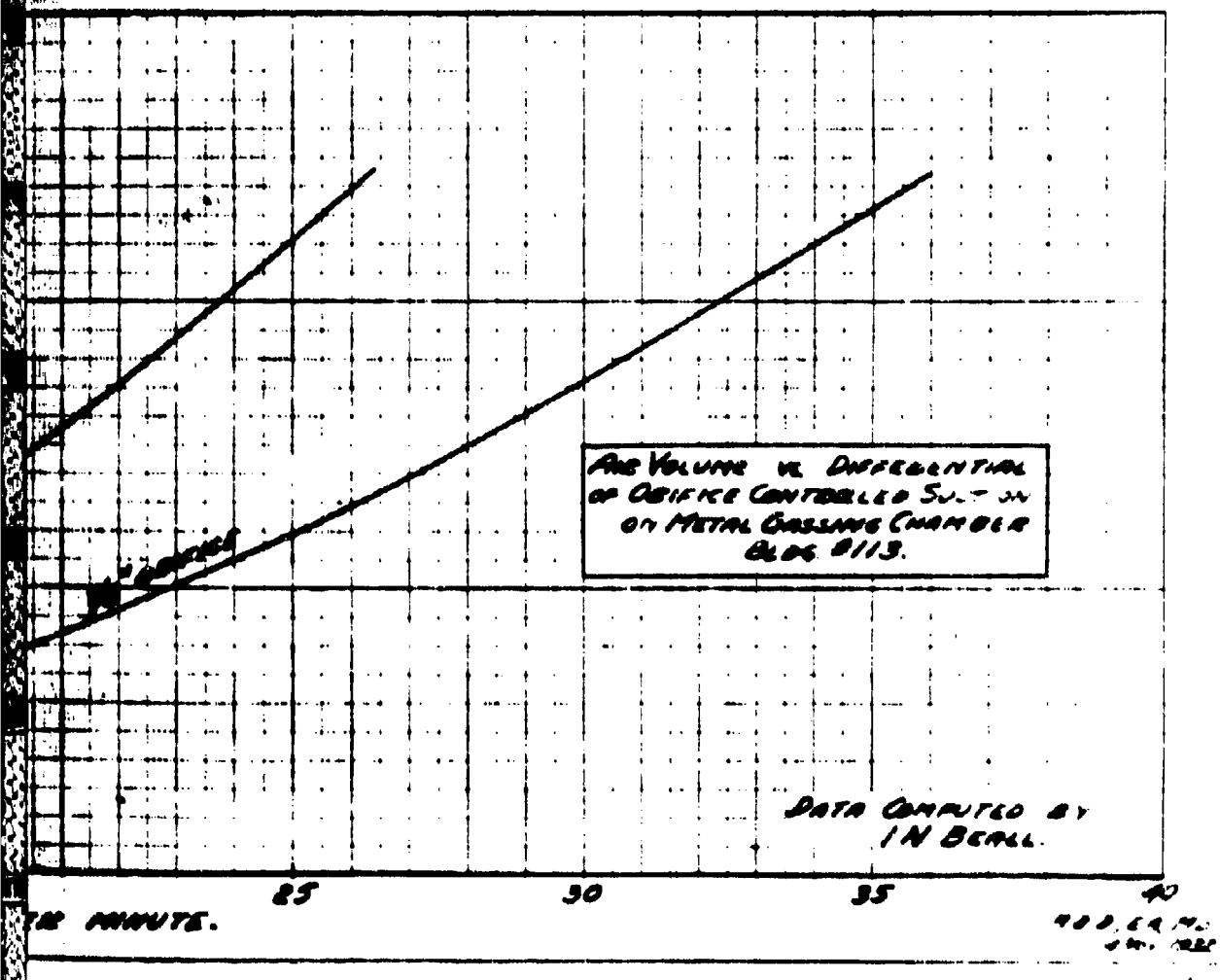

**Air-Suction Control Apparatus for Metal Chamber for Instantaneous Exposure of Mice.**

**OBJECT:** To show orifice set-up and equalizing tank.

**DESCRIPTION:** (1) Orifice plate. (2) Water manometer for measuring air flow through chamber. (3) Valve for regulating air flow. (4) Motor for operating pump for drawing air through chamber. Size "D" Leiman pump and 2-h.p. motor. (5) Equalizing tank. (6,6') Motor and pump for operating wet meter.

**PROJECT:** A 10.2.

**DATE:** Photographed at Edgewood Arsenal, Md., Nov. 28, 1932, for E.A.T.R. 119.


AIR VOLUME VS. DIFFERENTIAL
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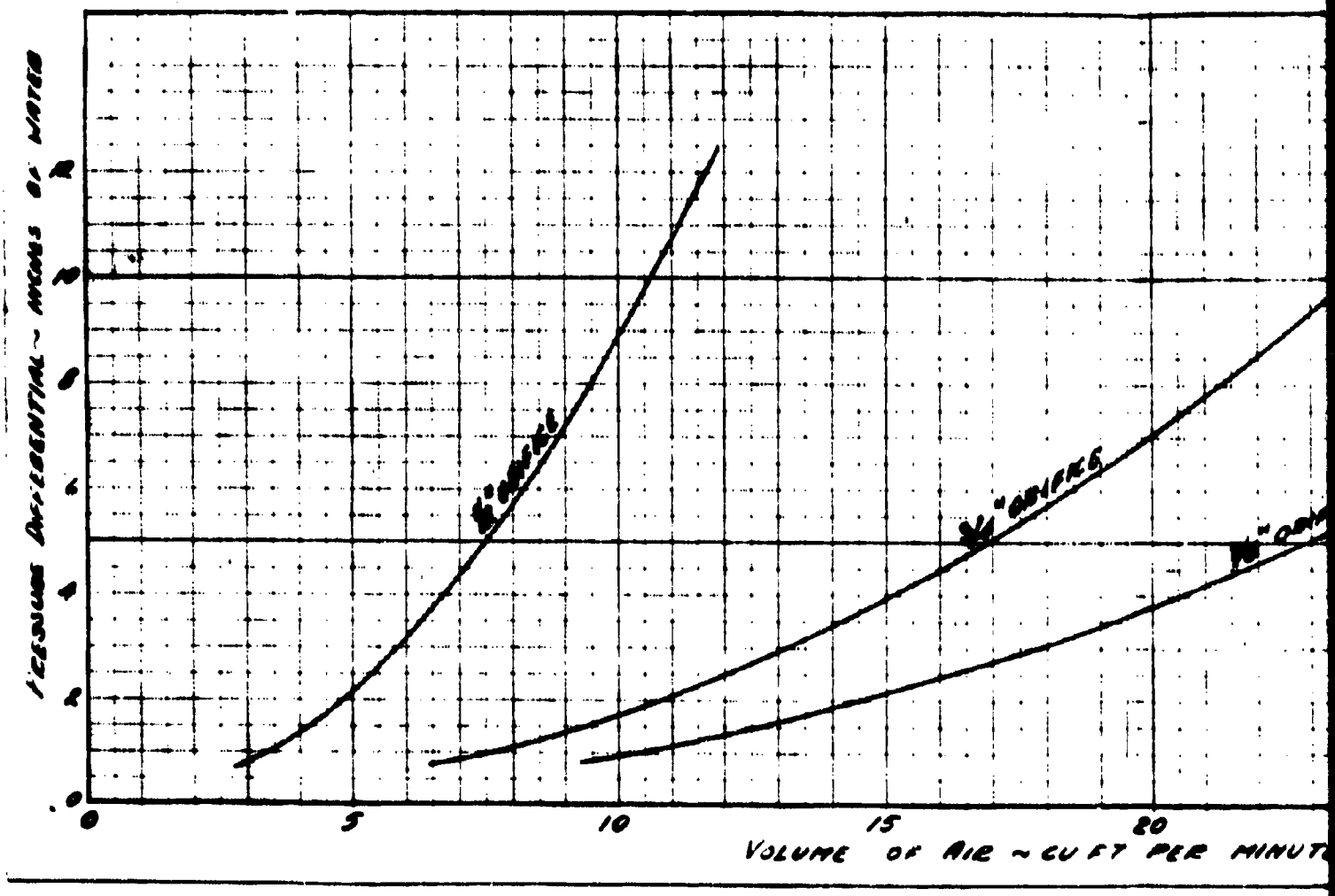
PRESSURE DIFFERENTIAL ~ INCHES OF WATER
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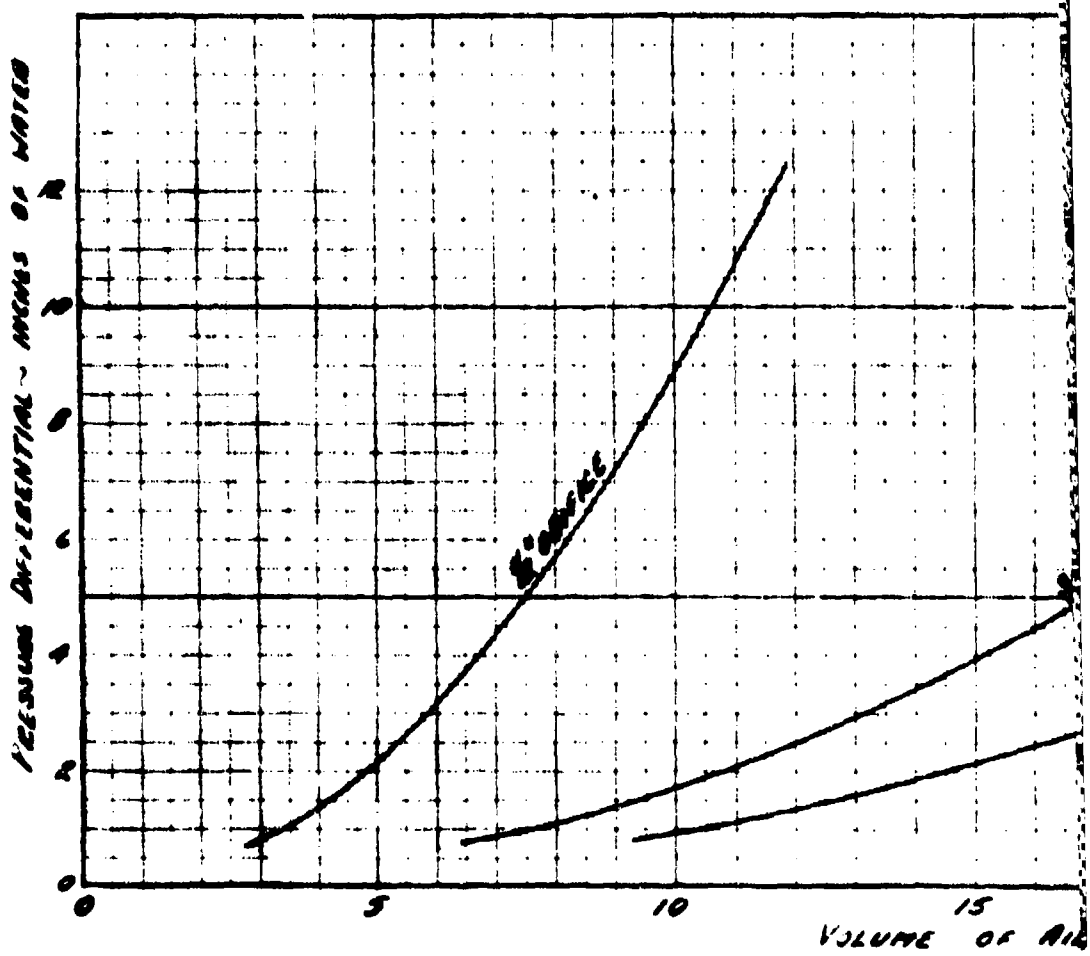
PRESSURE DIFFERENTIAL ~ INCHES OF WATER
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VOLUME OF AIR

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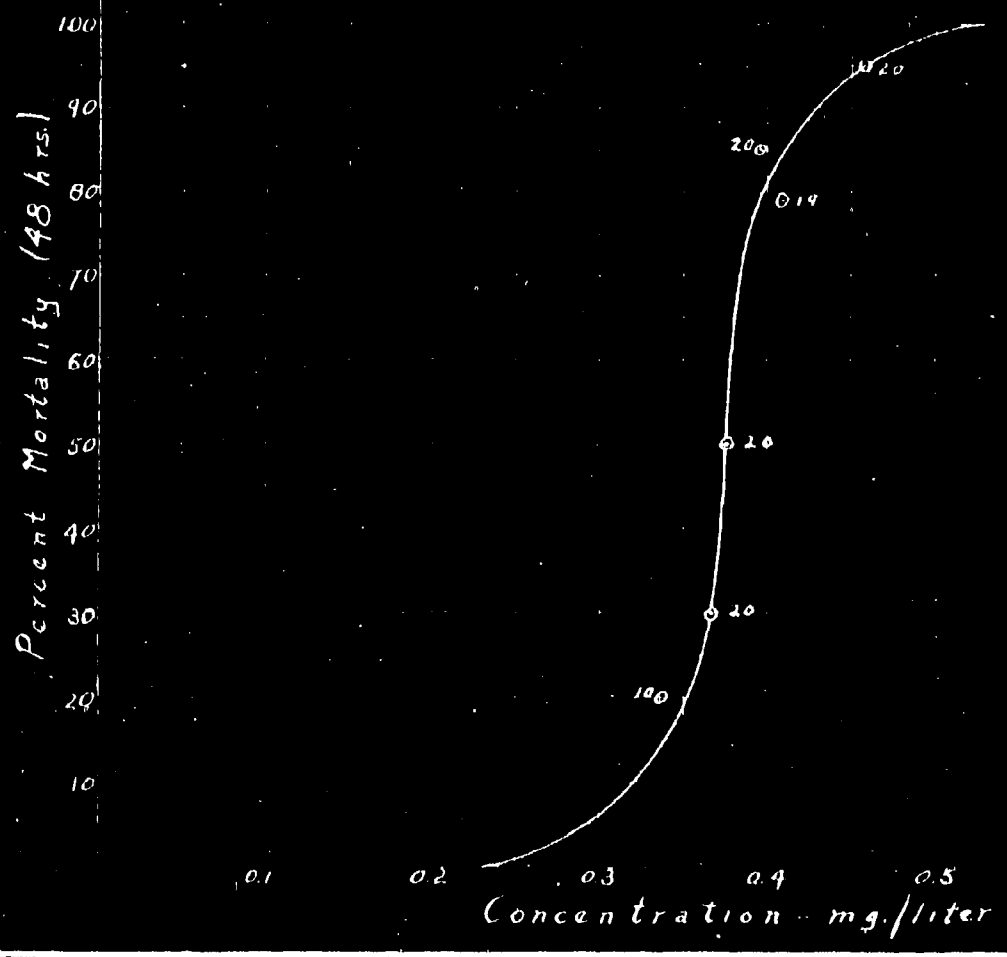
Graph
of
Toxicity of $CCl_4$
on
Mica
10 Minute Exposure
48 hour Mortality



**DEPARTMENT OF THE ARMY**
**US ARMY RESEARCH, DEVELOPMENT AND ENGINEERING COMMAND**
**EDGEWOOD CHEMICAL BIOLOGICAL CENTER**
**5183 BLACKHAWK ROAD**
**ABERDEEN PROVING GROUND, MD 21010-5424**

REPLY TO
ATTENTION OF

RDCB-DPC-RS

15 October 2015

MEMORANDUM THRU Director, Edgewood Chemical Biological Center, (RDCB-D/Dr. Joseph Corriveau), 5183 Blackhawk Road, Aberdeen Proving Ground, Maryland 21010-5424

FOR Defense Technical Information Center, 8725 John J. Kingman Road, Ft Belvoir, VA 22060

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1. This action is in response to an Edgewood Chemical Biological Center (ECBC) Internal Request for a Change in Distribution for the following documents as listed in attachment.

2. The listed documents have been reviewed by ECBC Subject Matter Experts and deemed suitable for the change in distribution to read "Approved for public release; distribution unlimited."

3. The point of contact is Adana Eilo, ECBC Security Specialist, (410) 436-2063 or adana.l.eilo.civ@mail.mil.

Encl

RONALD L. STAFFORD
Security Manager

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