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ATTN: DRSMC-CLJ-IR, Aberdeen Proving Ground, MD 21010-5423.

AUTHORITY

ECBC memo dtd 12 Dec 2017



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-DSR-S

DEC 12 2017

MEMORANDUM THRU Director, Edgewood Chemical Biological Center, (RDCB-D/ *ELM*
Dr. Eric L. Moore), 5183 Blackhawk Road, Aberdeen Proving Ground, MD 21010-5424

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

SUBJECT: Internal Request for Change in Distribution

1. This action is in response to an Edgewood Chemical Biological Center (ECBC) internal request for a Change in Distribution for the below listed documents, which are currently authorized for release to DoD Components Only:

a. ADB954958 - Report No. EAMRD 17; Project No., A3.2-3; Toxicity of Chlorine on Dogs- 10 minute exposure; Authors: G. C. Armstrong, W. A. Eldridge, J. A. Baldwin, dated 1 September 1923.

b. ADB958296 – Report No. EACD 328; Project No. C-4; Important Constants of Fourteen Common Chemical Warfare Agents; Author: C. H. Beebe; dated 1 December 1924.

c. ADB955268 – Report No. EATR 351; Project No. A 10.4; Chlorine. Median Lethal Concentration for Mice; Authors: S. D. Silver, F. P. McGrath; dated 9 May 1942.

d. ADB955266 – Report No. EATR 373; Project No. A 10.4; Chlorine. Median Lethal Concentration for Mice; Authors: S. D. Silver, F. P. McGrath, R. L. Ferguson; dated 17 July 1942.

2. The above listed documents have been reviewed by subject matter experts within ECBC and have been deemed suitable for the change in distribution to read "Approved for Public Release; Distribution Unlimited".

3. The point of contact is the undersigned at (410) 436-1999, ronald.l.stafford.civ@mail.mil.

for Adam Staff
RONALD L. STAFFORD
Security Manager

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EATR - 373

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(Project: A 10.4)

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CHLORINE.

Median Lethal Concentration for Mice.

DoD agencies and DoD contractors only. Each transmittal of this document outside the DoD and its contractors must have prior approval of:

By

S. D. Silver

F. P. McGrath

R. L. Ferguson

Commander

Chemical Research and Development Center

ATTN: DRSMC-CLJ-IR

Aberdeen Proving Ground, MD 21010

WAR DEPARTMENT
CHEMICAL WARFARE CENTER
EDGEWOOD ARSENAL, MD.

BRIGADIER GENERAL R. L. AVERY COMMANDING

COLONEL M. E. BARKER, CHIEF, RESEARCH & DEVELOPMENT DEPT.

COLONEL WM. D. FLEMING, CHIEF, MEDICAL RESEARCH DIVISION

by authority of *Special Agent*
John M. Adams
9 May 54
by

CW LABS, A Cml C. M.

DTIC
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Forwarded to Chief, Chemical Warfare Service,

JUL 17 1942

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E.A.T.R. 373

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CHLORINE.

Median Lethal Concentration for Mice.

By

S. D. Silver
F. P. McGrath
R. L. Ferguson

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CHLORINE. E D

Median Lethal Concentration for Mice.

ABSTRACT

Object.

The object of project A 10.4 is to collect "exact information on all those materials which may be indicated by the literature or by Chemical Warfare Service research as of possible interest for offensive chemical warfare agents".

The object of the work described in this report was to determine the median lethal concentration of chlorine for white mice for an exposure period of 10 min. and an observation period of 10 days. Directions regarding the work were given by the Chief, Chemical Warfare Service, as follows: "In order that a consistent basis of comparison can be maintained on all toxic compounds, it is desired that the median lethal concentration of chlorine for white mice on a 10-min. exposure be obtained experimentally and in sufficient detail to be reliable and that this value be used as a standard".*

Results.

Lethality to mice; Fifteen runs, using a 10-min. exposure period and an observation period of 10 days, gave the following results:

* 2nd ind., CWS 319.1/2183, C, CWS, April 12, 1941 to CG, EA, on letter, C, CWS, March 24, 1941, Project A 10.4, to CG, EA.

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ABSTRACT (Cont'd.)

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<u>Analytical concn. mg./l.</u>	<u>Fraction dead</u>	<u>Per cent deaths</u>
1.10	2/20	10
1.59	9/20	45
1.59	5/20	25
1.69	1/20	5
1.83	8/20	40
1.85	9/20	45
2.00	3/20	15
2.06	12/20	60
2.08	11/20	55
2.16	14/20	70
2.30	15/20	75
2.38	20/20	100
2.44	8/20	40
2.52	20/20	100
2.58	20/20	100

Conclusions.

The median lethal concentration of chlorine for selected male white mice of the Carworth Farms' CF-1 strain, for an exposure period of 10 min. and an observation period of 10 days, is 1.96 mg./l. with an average deviation for 12 runs of ± 0.19 mg./l. The majority of deaths were due to severe pulmonary edema.

Recommendations.

None.

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CHLORINE.

Median Lethal Concentration for Mice.

I. INTRODUCTION.

The object of project A 10.4 is to collect "exact information on all those materials which may be indicated by the literature or by Chemical Warfare Service research as of possible interest for offensive chemical warfare agents".

The object of the work described in this report was to determine the median lethal concentration of chlorine for male white mice of certain specified characteristics for an exposure period of 10 min. and an observation period of 10 days. The immediate purpose of these determinations was to secure results which are directly comparable with results obtained by the N.D.R.C. on the same strain of white mice. It was also desired to secure accurate data on chlorine with the idea of using chlorine as a standard of comparison for other toxic compounds.*

II. HISTORICAL.

The median lethal concentration of chlorine for mice was reported in E.A.T.R. 361 as 1.62, with an average deviation of ± 0.19 mg./l., for mice selected at random and 1.73, with an average deviation of ± 0.24 mg./l., for specially selected mice.

The University of Chicago Toxicity Laboratory has reported** the median lethal concentration of chlorine for mice of the same strain as those used in this report as 1.82 mg./l. with an average deviation of 0.20 mg. The mice used showed a 10-day death rate of 1%.

III. EXPERIMENTAL.

A. Material.

The chlorine used in the work described herein was obtained from the Toxic Gas Yard. Analysis by the Analytical Department of the Research Division showed it to be 99.97% pure.

* 2nd ind., CWS 319.1/2193, C, CWS, April 12, 1941 to CG, EA, on letter, C, CWS, March 24, 1941, Project A 10.4, to CG, EA.

** The Toxicity of Chlorine Gas for Mice, N.D.R.C. 131, Division B.

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B. Animals.

The mice used were purchased from the Carworth Farms under the following specifications: White (albino), all male, single uniform strain (Carworth Farms' CF-1 strain), 16 to 26 g. in weight, 5-1/2 to 6-1/2 weeks of age on receipt from dealer, good physical condition and appearance, free from parasitic or insect infection. These were purchased under the same specifications as used by the Y.D.R.C.

Three lots were used, namely, lots 34, 35, and 37. Twenty-five mice from each lot were set aside as controls to be observed daily in order to establish the daily death rate. Essential data are listed below.

Lot no.	Date rec'd.	Observation period	Daily death rate	Used in runs
		days	%	"
34	12/28/41	23	0.0	1-5
35	1/7/42	20	0.0	6-12
37	1/23/42	19	0.0	13-15

C. Apparatus.

Two new gassing chambers constructed of monel metal were installed in the rear rooms of bldg. 115. These were similar in design to the gassing chamber in the front room of the same building and described in E.A.T. reports 119 and 321. The volume of each chamber was 366 l. One of these was used in the work described herein.

The mouse cage and carrier used were identical with those described in E.A.T.R. 124. The apparatus for collection of gas-air samples from the chamber for analysis was the same as that described in E.A.T.R. 319. All apparatus is shown in photo. 8798, attached.

The airflow through the chamber was measured by an orifice meter in the effluent air line. This meter was calibrated by direct measurement of the airflow by means of a large dry meter placed in the line. The dry meter was manufactured by the American Meter Co., New York, N.Y., and was guaranteed by its make to have a maximum error of 1%. This method of calibration of the orifice meters used in measuring chamber airflows is described in detail in an E.A.T. report now in preparation.

D. Procedure.

Concentrations of chlorine were established in the gassing chamber by the same method as that described in E.A.T.R. 321. Briefly, the airflow through the chamber was first started and set at 250 l./min.

Then the chlorine was introduced into the chamber through a flowmeter which was maintained at a constant level by means of an equalizer tube. When the flowmeter reached the desired level a stopwatch was started.

From the calculation, $t_{99} = 4.6 \times \frac{\text{vol. of chamber}}{\text{airflow}}$, a 386-l. chamber requires 7.1 min. to reach 99% of constant concentration at an airflow of 250 l./min. Sampling was started after 3-1/2 min. had elapsed. The mice were introduced at the 10th min. and exposed for 10 min.

The interior of the chamber was protected from corrosion by being sprayed with a nonreactive lacquer composed of cellulose acetate dissolved in acetone and plasticized with triacetin.

Samples of the gas-air mixture, withdrawn from the gassing chamber for analysis at the rate of 1 l./min., were uniformly 10 l. as measured by the wet meter. The true volume of the sample was calculated from the barometric pressure and the meter manometer reading.

$$\text{True volume} = \frac{\text{Barometer (mm.)} - \text{Manometer (mm.)}}{\text{Barometer (mm.)}} \times 10$$

E. Nominal concentrations.

The capillary flowmeter for chlorine was calibrated by direct analysis of a timed sample. The setup described in E.A.T.R. 321 for calibration of flowmeters was used. Several levels of the flowmeter were checked in this way. The analytical results (in mg./min.) were divided by 250 l./min. (the airflow through the chamber) and the quotient, now expressed in mg. Cl₂/l., was plotted against the corresponding flowmeter heights. A smooth curve was drawn through these points and the nominal concentration for any height was read directly from the graph. This curve is shown in chart 2.

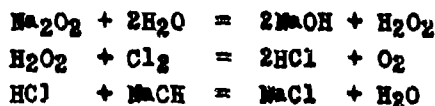
F. Analytical Concentration.

Samples of the gas-air mixture from the chamber were absorbed in 25 ml. of an 8% solution of sodium peroxide in water contained in one absorption tube. Experimental work showed that there was no necessity for a second absorption tube. After collection of the sample the solution was heated to boiling for 30 min., and the water lost by boiling was replaced. It was then cooled, and neutralized with 1:1 nitric acid using phenolphthalein as indicator. Then a few ml. excess of acid were added and chlorine was determined by the Volhard method. A definite volume of 0.02 N AgNO₃ was added and the solution was titrated with 0.02 N NaSCN using ferric alum as the indicator.

The concentration of chlorine was calculated by the use of the following formula;

$$\text{Anal. concn. (mg./l.)} = \frac{(\text{ml. AgNO}_3 - \text{ml. NaSCN})}{\text{corrected volume}} \times 0.71$$

The collection of chlorine in alkaline peroxide, yielding all the chlorine in the form of chlorides, may be expressed by the following equations*;



This method of analysis indicates total chlorine and is independent of hydrolysis of chlorine by water vapor in the air**.

G. Results.

The results of 15 runs using different concentrations of chlorine are shown in table 1. Twenty mice were used in each run. Runs are listed in order of increasing analytical concentration.

Table 1

Toxicity of Chlorine to Mice; 10-min. Exposure.

Run	Mouse lot	Date	Anal. concn. (mg./l.)	Temp. (°C.)	Rel. hum. (%)	Deaths (total, by days)										%
						1	2	3	4	5	6	7	8	9	10	
2	34	1/2	1.10	28	33	0	0	0	1	1	1	1	1	2	2	10
12	35	1/11	1.59	17	33	3	4	5	5	5	6	6	8	8	9	45
15	37	2/3	1.59	23	29	1	1	1	2	2	2	2	2	3	5	25
3	34	1/2	1.69	28	33	1	1	1	1	1	1	1	1	1	1	5
11	35	1/11	1.83	17	33	3	7	7	7	8	8	8	8	8	8	40
14	37	2/3	1.85	23	25	6	6	6	6	6	7	7	8	8	9	45
1	34	1/2	2.00	28	35	0	3	3	3	3	3	3	3	3	3	15
13	37	2/3	2.05	23	25	11	11	11	11	11	11	11	11	11	12	60
10	35	1/11	2.06	16	32	7	10	11	11	11	11	11	11	11	11	55
9	35	1/11	2.16	16	32	11	12	13	14	14	14	14	14	14	14	70
5	34	1/8	2.30	17	32	7	9	11	12	13	14	14	15	15	16	75
8	35	1/10	2.36	20	50	2	20	20	20	20	20	20	20	20	20	100
4	34	1/8	2.44	21	32	7	7	7	7	8	8	8	8	8	8	40
6	35	1/10	2.52	20	23	4	20	20	20	20	20	20	20	20	20	100
7	35	1/10	2.58	20	23	9	20	20	20	20	20	20	20	20	20	100

* Fries Ephraim, Inorganic Chemistry, 1939, p. 363.

** E.A.T.R. 321.

Concentration data (analytical) and total deaths in 10 days are plotted in chart 1 from which the median lethal concentration of chlorine is found to be 1.98 mg./l., with an average deviation for 12 runs of ± 0.19 .

Shortly after introduction into the gassing chamber the mice experienced severe irritation of the upper respiratory tract especially in the nasal passages. This caused a vigorous pawing of the nose and mouth, very often so violent as to break the skin and cause slight bleeding. The irritation soon passed to the lungs and was characterized by labored breathing or dyspnea, gasping, yawning, and spasmodic contraction of the intercostal muscles. In the final stages the mice went into marked convulsions and died shortly thereafter.

H. Pathology.

All of the mice dying in runs 13, 14, and 15 were autopsied and a pathological study made of their organs.

Deaths apparently could be the result of one of three causes. The first type showed a slight amount of edema in the lungs, accompanied by congestion. In the second, edema of the lungs seemed to be the predominating factor. The edema was patchy with a marked amount of congestion. In the third, a secondary pneumonia was present. The bronchioles and neighboring alveoli contained many polymorphonuclear leucocytes, debris and fibrin.

The animals from the three above-mentioned runs which survived the 10-day observation period were killed and also examined under the microscope. In every case the lungs were practically free from pathological changes.

The distribution of the types of death showed that the great preponderance of the chlorine deaths were due to severe edema and congestion (second type mentioned above). There was only one death due to mild edema and congestion (first type, above) and but two due to pneumonia. There was no particular correlation between the type of death and the time of death although most of the total deaths, and therefore most of the severe edema deaths, occurred during the first 24 hr. after exposure.

IV. DISCUSSION.

A. Concentration Data.

The agreement between the nominal and the analytical concentrations is shown in table 2.

Table 2

Comparison of Nominal and Analytical Concentrations
of Chlorine.

Run	Concentration		C_a/C_n
	Nominal	Analytical	
	Mg./l.	Mg./l.	
1	2.12	2.00	0.94
2	1.24	1.10	0.89
3	1.83	1.69	0.92
4	2.52	2.44	0.97
5	2.32	2.30	0.99
6	2.58	2.52	0.94
7	2.52	2.58	1.02
8	2.42	2.36	0.97
9	2.18	2.16	0.99
10	2.00	2.06	1.03
11	1.78	1.83	1.03
12	1.58	1.59	1.01
13	2.11	2.06	0.97
14	1.88	1.85	0.98
15	1.70	1.69	0.94

For the fifteen runs the analytical concentrations average 97.3% of the nominal concentrations. Analytical concentrations were used for the computation of the median lethal concentration for mice.

Sources of experimental error are tabulated below:

<u>Source of error.</u>	<u>Percent error</u>
1. <u>Chemical and physical.</u>	
a. <u>Compound</u>	
Parity 99.97%	0.03
b. <u>Analytical concentration, $c_a = m/v$</u>	
m = total milligrams found, depending on titrating solution	
ml. $AgNO_3$ used near m.l.c. = 4 + 0.02 ml.	0.5
v = volume of sample, measured on corrected meter = 10 ± 0.01 l.	0.1

Percent error

c. Nominal concentration, $C_N = R/Q$

R = weight loss/time, measured on a
flowmeter, accuracy about 4.0

Q = airflow through chamber 4.0

2. Biologic.

a. Animals

Physiologic variations due to age, sex,
former nutritional history, etc. Indeterminable

B. Median Lethal Concentration.

The median lethal concentration of chlorine for mice as found in this work (1.9%, with an average deviation of ± 0.19 mg./l.) is in good agreement with that found by the Chicago Laboratory of the N.D.R.C. (1.82, with an average deviation of ± 0.2 mg./l.).

The average deviation of the runs from the line (± 0.19) appears at first glance to be quite large, but on comparison with the m.l.c. it is actually less than 10% of the latter, indicating a fair degree of internal consistency of the runs made.

The difference in the control death rates (0.0% in 10 days for Edgewood Arsenal as compared to 1% in 10 days for the Chicago laboratory) might be an indication of the differences in resistance of the mice at the two laboratories which made the Edgewood Arsenal m.l.c. the higher of the two.

Deaths occurred mostly within the first 24 hr. after gassing, continuing at a lesser rate until 48 hr. after gassing, then were only sporadic for the remainder of the 10-day observation period.

The work described in this report was carried out on male mice of the CF-1 strain, purchased from the Carworth Farms. Earlier reported work on chlorine, E.A.T.R. 351, carried out on healthy mice with no regard to sex ratio, when compared with present results give rise to an interesting speculation based on the following:

(a) When the mice used were selected for one strain with no regard to sex ratio the m.l.c. of chlorine was 1.52 mg./l. with an average deviation for 20 runs of ± 0.19 mg./l.

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(b) When a second series of runs on chlorine was made using 10 runs of the original strain of mice (same as in (a) above), and 5 runs of the CF-1 strain (half males and half females), an m.l.c. of 1.73 mg./l. with an average deviation for 20 runs of ± 0.24 mg./l. was obtained.

(c) When another laboratory (University of Chicago Toxicity Laboratory) using male mice of still another strain determined the m.l.c. for chlorine, the figure obtained was 1.82 mg./l. with an average deviation for 10 runs of ± 0.20 .

(d) The median lethal concentration of chlorine using male mice of the CF-1 strain was shown in this report to be 1.96 with an average deviation for 15 runs of ± 0.19 .

These results would seem to indicate that the m.l.c. for chlorine on male mice is higher than on a mixture of male and female mice. This tends to corroborate unpublished findings at this laboratory.

V. CONCLUSIONS.

The median lethal concentration of chlorine for selected male white mice of the Carworth Farms' CF-1 strain, for an exposure period of 10 min. and an observation period of 10 days, is 1.96 mg./l. with an average deviation for 12 runs of ± 0.19 mg./l. The majority of deaths were due to severe pulmonary edema.

VI. RECOMMENDATIONS.

None.

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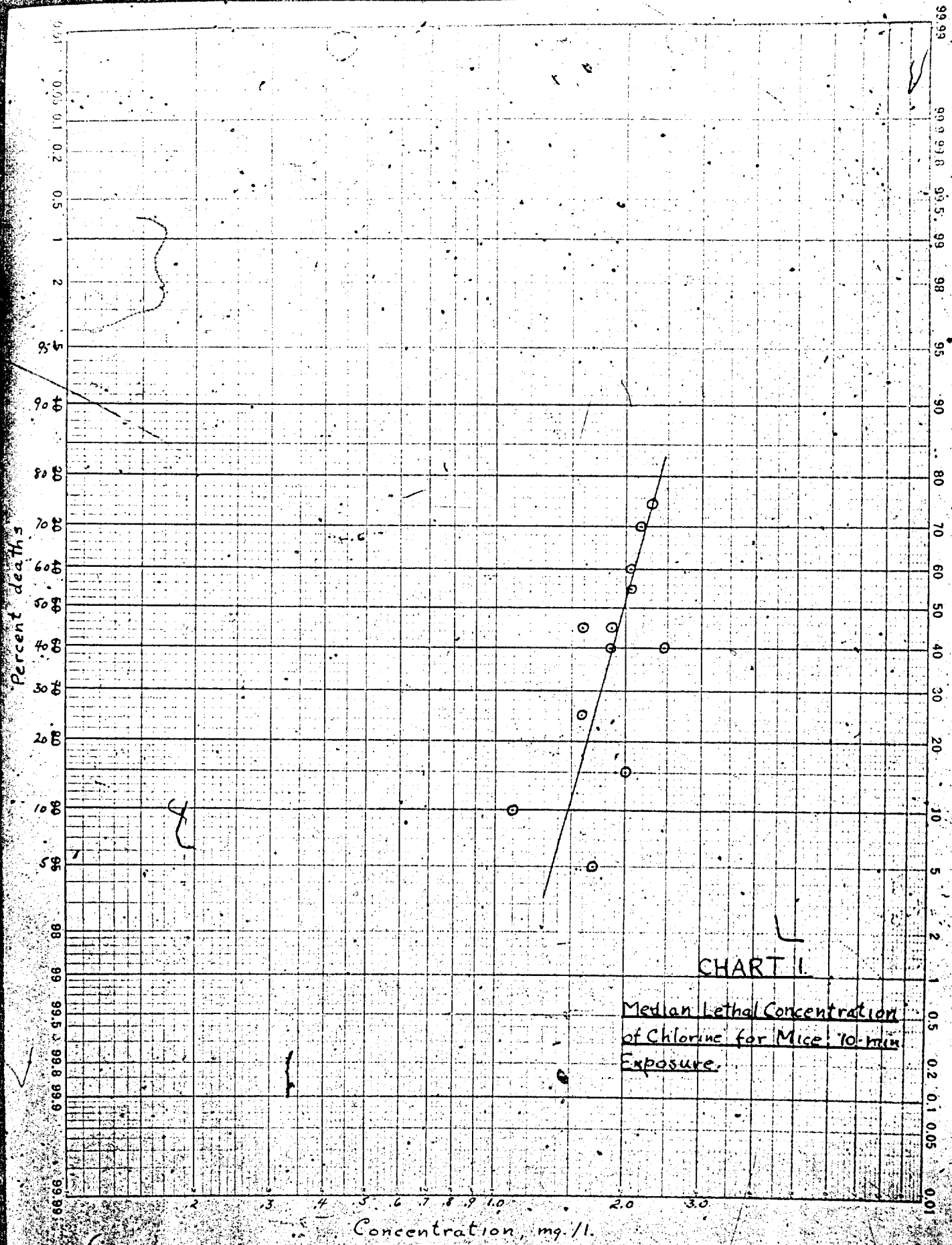


CHART I

Median Lethal Concentration
of Chlorine for Mice, 10-min
Exposure.

Concentration, mg/l.

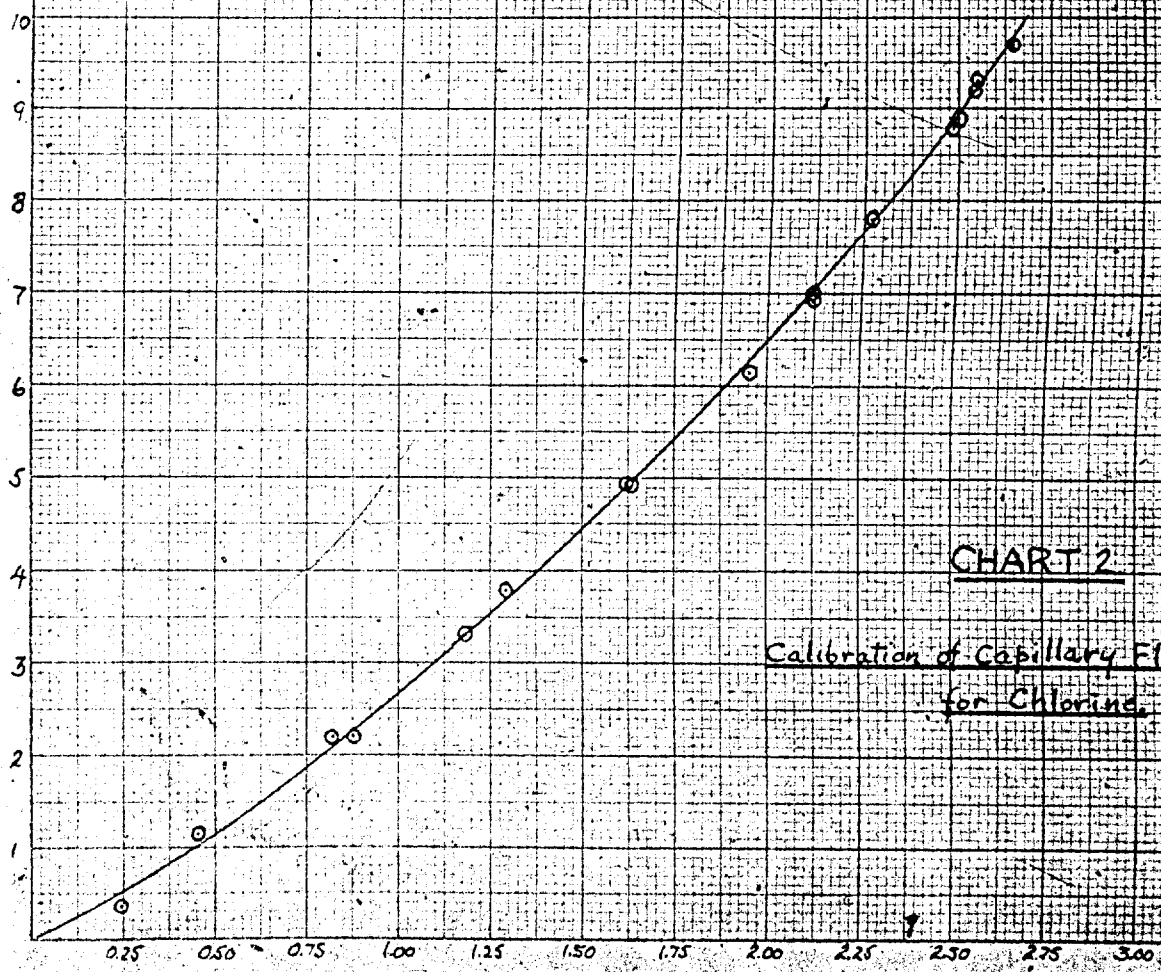
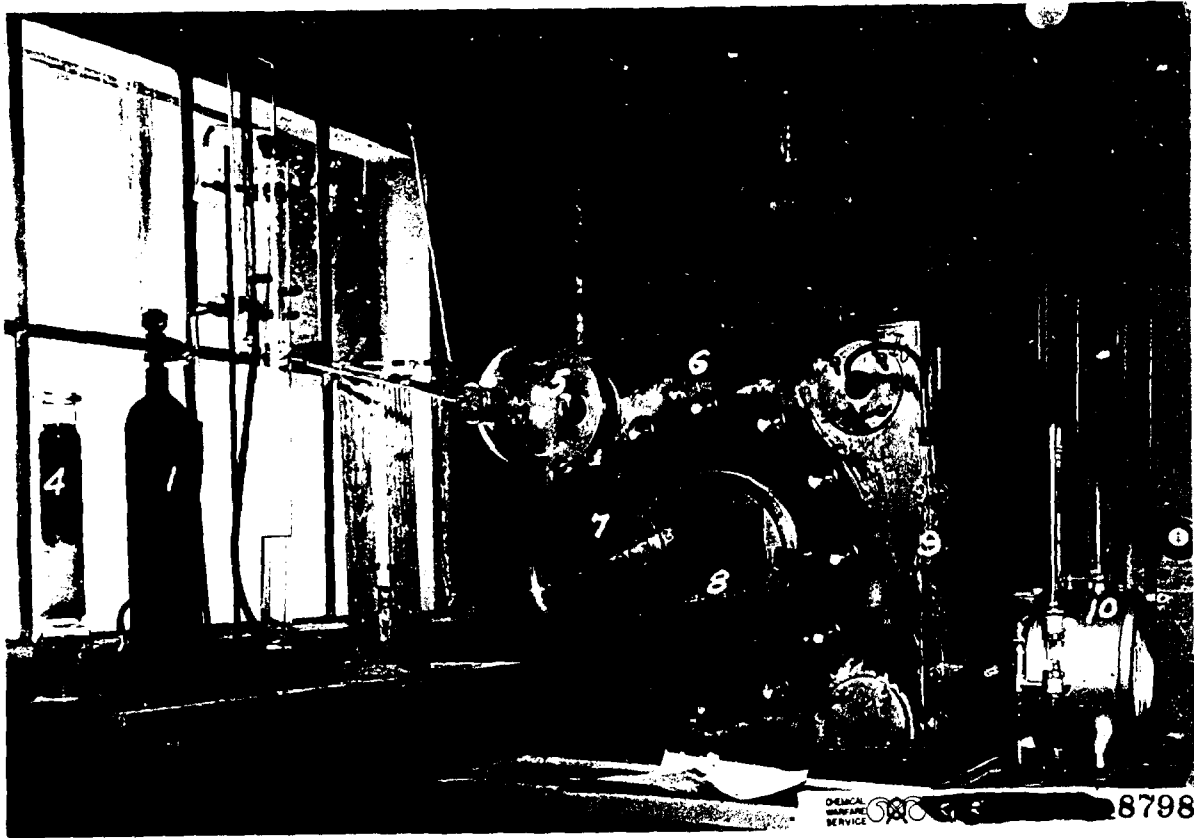


CHART 2

Calibration of Capillary Flowmeter
for Chlorine



Apparatus Used for Determination of Median Lethal Concentration of Chlorine for Mice.

Object: To show setup of apparatus.

Description:

1. Cylinder containing chlorine.
2. Pressure equalizer tube.
3. Chlorine flowmeter.
4. Tower containing absorbent for excess chlorine from equalizer tube.
5. Glass mixing bowl.
6. Gassing chamber.
7. Mouse-cage carriage.
8. Twenty-compartment mouse cage.
9. Sample absorbing bubbler.
10. Wet meter equipped with thermometer and manometer, for measuring gas-air sample volume.

Project: A 10.4, Collection of Toxicity Data.

Date: Photographed at Edgewood Arsenal, July 9, 1941, for use in technical reports.

Submitted:

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CHLORINE.
Median Lethal Concentration for
Mice.

Experimental work:

Started: January 2, 1942
Completed: February 8, 1942
Notebook: 792

E.A.T.R. 378.

Project: A 10.4

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Forwarded, July 17, 1942.

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