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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](mailto:ronald.l.stafford.civ@mail.mil).

*for Adam Staff*  
RONALD L. STAFFORD  
Security Manager

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EATR 351

E.A.T.R. 351, COPY NO. 1.

(Project: A 10.4)

CHLORINE.  
MEDIAN LETHAL CONCENTRATION FOR MICE.

By

S. D. Silver  
F. P. McGrath

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Commander  
Chemical Research and Development Center  
ATTN: DRSMC-CLJ-IR  
Aberdeen Proving Ground, MD 21010

WAR DEPARTMENT  
CHEMICAL WARFARE SERVICE  
EDGEWOOD ARSENAL, MD.

BRIGADIER GENERAL R. L. AVERY, COMMANDING

COLONEL M. E. BARKER, TECHNICAL DIRECTOR

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

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Forwarded to Chief, Chemical Warfare Service,

MAY 9 1942

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An earlier edition of this report, dated Sept. 23, 1941, has been withdrawn and destroyed.

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CHLORINE.  
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By

S. D. Silver  
F. P. McGrath

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CHLORINE.  
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 of possible interest for offensive chemical warfare agents as indicated either by the literature or by Chemical Warfare Service research.

The object of the work described in this report was to determine the median lethal concentration of chlorine for white mice exposed for 10 minutes and observed for 10 days, in order to establish a standard with which similar data on other compounds may be compared.

Results.

Lethality to mice: Forty-nine runs, using a 10-min. exposure period and an observation period of 10 days, at concentrations from 0.73 to 3.30 mg./l. are reported in table 1, pages 5 and 6.

Conclusions.

1. When the mice used were selected at random from miscellaneous lots, the m.l.c. of chlorine for an exposure period of 10 min. and an observation period of 10 days was  $1.52 \pm$  an average deviation (for 20 runs of 20 mice each) of 0.19 mg./l.

2. When mice of carefully selected strain having a daily death rate for 10 days of 0.0% were used the m.l.c. of chlorine for an exposure period of 10 min. and an observation period of 10 days was  $1.73 \pm$  an average deviation for 20 runs of 0.24 mg./l.

Recommendation.

None, since further work is in progress and will be reported in E.A.T.R. 373.

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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 of possible interest for offensive chemical warfare agents as indicated either by the literature or by Chemical Warfare Service research.

The object of the work described in this report was to determine the median lethal concentration of chlorine for white mice exposed for 10 minutes and observed for 10 days, in order to establish a standard with which similar data on other compounds may be compared.

II. HISTORICAL.

No record has been found of any earlier work done to determine the median lethal concentration of chlorine for mice.

Runs 1 to 33, inclusive, of this report were first reported in the original issue of E.A.T.R. 351, now superseded by the present report. When certain errors were discovered in the method of analysis used in runs 1 to 16, namely, the assumption of complete conversion of chlorine to chlorides in sodium hydroxide solution, the original scheme of analysis was checked in Medical Research Division.

It was found that only 5/6 of the chlorine (83.3%) was converted to the chloride. The analytical concentrations of runs 1 to 16, inclusive, have been recalculated on the basis of this fact and have been incorporated in the present report. Additional work is also reported.

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.

B. Animals.

The mice used were purchased from dealers. Seven separate lots were used, namely, lots 13, 15, 18, 19, 20, 30, and 31. Twenty-five

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mice from each lot were set aside as controls to be observed daily in order to establish the normal daily death rates. The essential data are listed below.

| <u>Lot no.</u> | <u>Total days observed</u> | <u>Normal daily death rate</u><br>% | <u>Normal 10-day death rate</u> | <u>Used in run</u> |
|----------------|----------------------------|-------------------------------------|---------------------------------|--------------------|
| 13             | 16                         | 0.0                                 | 0.0                             | 1-11               |
| 15             | 20                         | 1.4                                 | 14.                             | 12-16              |
| 19             | 14                         | 0.0                                 | 0.0                             | 17-27              |
| 18             | 24                         | 0.17                                | 1.7                             | 28-29              |
| 20             | 14                         | 0.29                                | 2.9                             | 30-33              |
| 30             | 32                         | 0.0                                 | 0.0                             | 34-44              |
| 31             | 15                         | 0.0                                 | 0.0                             | 45-49              |

Lot 30, and subsequent lots, were bought on new specifications limiting weight between 18 and 25 grams. Lot 31 was half males and half females. This lot was purchased from Carworth Farms and was designated as their CF-1 strain.

#### C. Apparatus.

Two new gassing chambers constructed of monel metal were installed in the rear rooms of bldg. 113. 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 386 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.

#### 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 air flow 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 equation,  $t_{99} = 4.6 \times \frac{\text{vol. of chamber}}{\text{air flow}}$ , a 386-l. chamber requires 7.1 min. to reach 99% of constant concentration at an air flow

of 250 l./min. Accordingly, sampling was started after 8-1/2 min. had elapsed. The mice were introduced at the 10th min. and exposed for 10 min.

The flowmeter used in runs 1 to 16, inclusive, was replaced in runs 17 to 49 by another with a smaller range in an effort to increase the ease of establishment of the chlorine concentrations.

Examination of the interior of the chamber after the 17th run showed the presence of considerable quantities of nickel chloride evidently due to the corrosive action of the chlorine on the monel metal of the chamber walls. This deposit was cleaned off and the chamber sprayed with a lacquer composed of cellulose acetate dissolved in acetone and plasticized with triacetin. This treatment stopped further corrosion until run 30, from which point, until run 33, there was a slight renewal of attack. The chamber was relacquered for runs 34 to 49.

Samples of the gas-air mixture, withdrawn from the gassing chamber for analysis at the rate of 1 l./min., were uniformly 10 l. on the wet meter. The true volume 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 Concentration.

The nominal concentrations were calculated from the chlorine flowmeter readings and the air flow through the chamber. In runs 1 to 16, inclusive, the flowmeter utilized was one manufactured by the Corning Glass Co. It had an adjustment by which any one of four different orifices could be used. The orifice approximated a true plate orifice and therefore the following method was used for the calculation of chlorine flows:

The flowmeter was first calibrated by passing pure nitrogen through it to a wet meter and reading the actual flow in ml./min. for seven different levels of the flowmeter. A stopwatch was used for timing each operation and each level was checked twice. By an application of the diffusion law the flow for each level was multiplied by the square root of the ratio of the molecular weight of nitrogen to the molecular weight of chlorine. This factor was computed to be 0.628. The flow was thus found in terms of ml. of chlorine per minute and each flow was plotted against the corresponding flowmeter height and a smooth curve drawn.

By dividing the molecular weight of chlorine by 22.4 liters and correcting to the temperature and pressure of the experiment the density

of the chlorine was calculated for each run. The nominal concentration of chlorine was then found from the following:

$$\text{Nominal concn. (mg./l.)} = \frac{\text{ml. Cl/min. (from flowmeter height)} \times \text{density (mg./ml.)}}{\text{air flow through chamber (l./min.)}}$$

In runs 17 to 49 a capillary type of flowmeter was used, and data could not be so easily translated from terms of nitrogen to terms of chlorine by calculation. It was therefore decided to calibrate this flowmeter by direct analysis of a timed sample. The setup described in E.A.T.R. 321 for calibration of the chlorine flowmeter 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 air flow through the chamber) and the quotient, now expressed in mg. Cl/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. The curve was extrapolated to a flowmeter height of 9 inches. This curve is shown in chart 2.

#### F. Analytical Concentration.

In runs 1-16, inclusive, the samples were absorbed in 25 ml. of 10% NaOH. Experimental work showed there was no necessity for a second bubbler. This solution was 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 as follows. A definite volume of 0.0999 N  $\text{AgNO}_3$  was added and the solution was titrated with 0.1189 N NaSCN, using ferric alum as an indicator.

This method is outlined in Scott: Standard Methods of Chemical Analysis, 5th Ed., 1939, p. 268. However, in order to determine whether chlorine is converted completely to chlorides by sodium hydroxide solutions, this scheme of analysis was checked in the Medical Research Division, using known concentrations of freshly prepared chlorine water. It was found that only 5/6 of the chlorine (83.3%) was converted to the chloride. The concentrations found by this method were therefore divided by 0.833 to obtain the true analytical concentrations.

In runs 17-49, inclusive, the gas-air mixture was absorbed in 25 ml. of an 8% solution of sodium peroxide in water. The chlorine is reduced quantitatively to chlorides by the  $\text{H}_2\text{O}_2$  produced when  $\text{Na}_2\text{O}_2$  is dissolved in water. This solution was then heated to boiling for 30 min., and the water lost by boiling was replaced. It was then allowed to cool, after which it was put through the same procedure as the samples from runs

1-16. This method is not described in detail in the literature but is the standard method used in the analytical laboratory at this Arsenal.

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

$$\text{Anal. concn. (mg./l.)} = \frac{[(\text{NF}^* \text{AgNO}_3 \times \text{ml. AgNO}_3) - (\text{NF}^* \text{NaSCN} \times \text{ml. NaSCN})]}{\text{corrected volume}} \times 35.5$$

Runs 1 to 33, inclusive, were made in the original investigation. When certain errors were discovered in this work, including the error of assuming a complete conversion of chlorine to chlorides in the method of analysis first described above, it was decided to make additional runs to insure more accurate results. Runs 34 to 49 were made for this purpose.

### G. Results.

The results of 49 runs using different concentrations of chlorine are shown in table 1. Twenty mice were used in each run except in four instances, runs 35-38, where 40 mice were used. Runs are listed in order of increasing analytical concentration.

Table 1

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

| Run | : Mouse | : lot   | : Date | : Anal. :<br>: concn. : | : Temp. :<br>: °C. : | : Rel. :<br>: hum. : | Deaths (total. by days) |      |      |      |      |      |      |      |      |      |     |   |   |   |
|-----|---------|---------|--------|-------------------------|----------------------|----------------------|-------------------------|------|------|------|------|------|------|------|------|------|-----|---|---|---|
|     |         |         |        |                         |                      |                      | 1                       | 2    | 3    | 4    | 5    | 6    | 7    | 8    | 9    | 10   |     |   |   |   |
|     |         |         |        | : 1941:mg./l.:          | : °C. :              | : % :                | :                       | :    | :    | :    | :    | :    | :    | :    | :    | :    | :   | : | : | : |
| 10  | : 13    | : 4/23  | : 0.73 | : 22                    | : 50                 | : 0                  | : 0                     | : 0  | : 0  | : 0  | : 0  | : 1  | : 1  | : 2  | : 2  | : 10 |     |   |   |   |
| 33  | : 20    | : 6/16  | : 0.84 | : 27                    | : 72                 | : 0                  | : 0                     | : 0  | : 1  | : 1  | : 1  | : 1  | : 1  | : 1  | : 1  | : 5  |     |   |   |   |
| 29  | : 18    | : 6/10  | : 0.92 | : 26                    | : 50                 | : 0                  | : 0                     | : 0  | : 0  | : 0  | : 0  | : 0  | : 0  | : 0  | : 0  | : 1  | : 5 |   |   |   |
| 34  | : 30    | : 10/30 | : 1.00 | : 27                    | : 44                 | : 1                  | : 1                     | : 1  | : 1  | : 1  | : 1  | : 1  | : 2  | : 2  | : 4  | : 20 |     |   |   |   |
| 9   | : 13    | : 4/23  | : 1.02 | : 21                    | : 50                 | : 0                  | : 0                     | : 0  | : 0  | : 0  | : 0  | : 0  | : 0  | : 0  | : 0  | : 0  | : 0 |   |   |   |
| 11  | : 13    | : 4/23  | : 1.13 | : 22                    | : 50                 | : 2                  | : 2                     | : 2  | : 2  | : 2  | : 2  | : 2  | : 2  | : 3  | : 3  | : 15 |     |   |   |   |
| 12  | : 15    | : 5/6   | : 1.15 | : 27                    | : 54                 | : 1                  | : 1                     | : 1  | : 2  | : 2  | : 3  | : 7  | : 7  | : 7  | : 7  | : 35 |     |   |   |   |
| 35* | : 30    | : 10/30 | : 1.24 | : 27                    | : 44                 | : 2                  | : 2                     | : 4  | : 8  | : 9  | : 9  | : 9  | : 9  | : 10 | : 13 | : 33 |     |   |   |   |
| 32  | : 20    | : 6/16  | : 1.34 | : 27                    | : 68                 | : 4                  | : 5                     | : 5  | : 5  | : 5  | : 6  | : 7  | : 7  | : 8  | : 8  | : 40 |     |   |   |   |
| 40  | : 30    | : 11/12 | : 1.35 | : 19                    | : 50                 | : 1                  | : 1                     | : 1  | : 1  | : 1  | : 1  | : 1  | : 4  | : 6  | : 11 | : 55 |     |   |   |   |
| 31  | : 20    | : 6/16  | : 1.40 | : 27                    | : 68                 | : 6                  | : 11                    | : 11 | : 12 | : 12 | : 12 | : 12 | : 12 | : 12 | : 12 | : 60 |     |   |   |   |
| 13  | : 15    | : 5/6   | : 1.42 | : 27                    | : 54                 | : 2                  | : 2                     | : 2  | : 2  | : 2  | : 3  | : 5  | : 5  | : 5  | : 5  | : 25 |     |   |   |   |
| 8   | : 13    | : 4/23  | : 1.43 | : 21                    | : 50                 | : 1                  | : 1                     | : 1  | : 1  | : 1  | : 1  | : 1  | : 1  | : 1  | : 2  | : 10 |     |   |   |   |

\* NF = normality factor.

Table 1 (Cont'd.)

| Run | : Mouse : | Date :  | Anal. :       | Temp. : | Rel. : | Deaths (total, by days) |            |          |      |      |      |      |      |      |      |      |     |      |
|-----|-----------|---------|---------------|---------|--------|-------------------------|------------|----------|------|------|------|------|------|------|------|------|-----|------|
|     |           |         |               |         |        | : lot :                 | : concn. : | : hum. : | 1 :  | 2 :  | 3 :  | 4 :  | 5 :  | 6 :  | 7 :  | 8 :  | 9 : | 10 : |
|     |           |         | 1941:mg./l. : | °C. :   | % :    |                         |            |          |      |      |      |      |      |      |      |      |     | %    |
| 28  | : 18 :    | 6/10 :  | 1.43 :        | 26 :    | 50 :   | 8 :                     | 11 :       | 11 :     | 11 : | 11 : | 11 : | 11 : | 12 : | 12 : | 12 : | 12 : | 60  |      |
| 36* | : 30 :    | 10/30 : | 1.47 :        | 28 :    | 44 :   | 2 :                     | 2 :        | 2 :      | 2 :  | 2 :  | 3 :  | 4 :  | 8 :  | 9 :  | 14 : | 35   |     |      |
| 27  | : 19 :    | 6/10 :  | 1.47 :        | 26 :    | 50 :   | 10 :                    | 11 :       | 11 :     | 11 : | 11 : | 12 : | 12 : | 12 : | 12 : | 12 : | 60   |     |      |
| 45  | : 31 :    | 11/18 : | 1.57 :        | 24 :    | 39 :   | 0 :                     | 0 :        | 0 :      | 0 :  | 0 :  | 0 :  | 0 :  | 0 :  | 1 :  | 2 :  | 10   |     |      |
| 37* | : 30 :    | 10/30 : | 1.62 :        | 30 :    | 44 :   | 4 :                     | 4 :        | 4 :      | 4 :  | 4 :  | 4 :  | 7 :  | 9 :  | 14 : | 16 : | 40   |     |      |
| 41  | : 30 :    | 11/12 : | 1.62 :        | 20 :    | 50 :   | 5 :                     | 5 :        | 5 :      | 5 :  | 5 :  | 5 :  | 5 :  | 7 :  | 9 :  | 11 : | 55   |     |      |
| 16  | : 15 :    | 5/7 :   | 1.65 :        | 22 :    | 61 :   | 5 :                     | 6 :        | 6 :      | 6 :  | 6 :  | 7 :  | 7 :  | 7 :  | 8 :  | 8 :  | 40   |     |      |
| 26  | : 19 :    | 6/10 :  | 1.69 :        | 26 :    | 50 :   | 18 :                    | 18 :       | 18 :     | 19 : | 19 : | 20 : | 20 : | 20 : | 20 : | 20 : | 100  |     |      |
| 14  | : 15 :    | 5/7 :   | 1.72 :        | 25 :    | 54 :   | 2 :                     | 3 :        | 4 :      | 4 :  | 5 :  | 8 :  | 12 : | 12 : | 13 : | 13 : | 65   |     |      |
| 30  | : 20 :    | 6/16 :  | 1.72 :        | 25 :    | 78 :   | 16 :                    | 17 :       | 18 :     | 18 : | 18 : | 18 : | 18 : | 18 : | 18 : | 18 : | 90   |     |      |
| 42  | : 30 :    | 11/12 : | 1.73 :        | 21 :    | 52 :   | 7 :                     | 7 :        | 7 :      | 7 :  | 8 :  | 9 :  | 10 : | 10 : | 10 : | 11 : | 55   |     |      |
| 46  | : 31 :    | 11/18 : | 1.73 :        | 24 :    | 39 :   | 1 :                     | 1 :        | 1 :      | 1 :  | 1 :  | 1 :  | 1 :  | 3 :  | 3 :  | 5 :  | 25   |     |      |
| 15  | : 15 :    | 5/7 :   | 1.74 :        | 24 :    | 69 :   | 10 :                    | 10 :       | 12 :     | 12 : | 14 : | 17 : | 18 : | 18 : | 18 : | 18 : | 90   |     |      |
| 47  | : 31 :    | 11/18 : | 1.82 :        | 25 :    | 39 :   | 3 :                     | 3 :        | 3 :      | 3 :  | 3 :  | 3 :  | 3 :  | 4 :  | 4 :  | 6 :  | 30   |     |      |
| 38* | : 30 :    | 10/30 : | 1.83 :        | 30 :    | 35 :   | 7 :                     | 7 :        | 7 :      | 7 :  | 8 :  | 8 :  | 10 : | 12 : | 13 : | 15 : | 38   |     |      |
| 7   | : 13 :    | 4/23 :  | 1.85 :        | 20 :    | 50 :   | 14 :                    | 15 :       | 15 :     | 15 : | 15 : | 15 : | 15 : | 15 : | 15 : | 16 : | 80   |     |      |
| 33  | : 30 :    | 10/30 : | 1.87 :        | 32 :    | 33 :   | 11 :                    | 12 :       | 12 :     | 12 : | 12 : | 13 : | 14 : | 16 : | 17 : | 18 : | 90   |     |      |
| 43  | : 30 :    | 11/12 : | 1.89 :        | 21 :    | 46 :   | 9 :                     | 10 :       | 10 :     | 11 : | 11 : | 11 : | 12 : | 12 : | 13 : | 14 : | 70   |     |      |
| 25  | : 19 :    | 6/10 :  | 1.99 :        | 24 :    | 54 :   | 13 :                    | 13 :       | 13 :     | 13 : | 14 : | 15 : | 15 : | 15 : | 15 : | 15 : | 75   |     |      |
| 44  | : 30 :    | 11/12 : | 2.02 :        | 21 :    | 46 :   | 6 :                     | 6 :        | 6 :      | 7 :  | 7 :  | 7 :  | 8 :  | 9 :  | 9 :  | 10 : | 50   |     |      |
| 48  | : 31 :    | 11/12 : | 2.03 :        | 25 :    | 32 :   | 5 :                     | 5 :        | 5 :      | 5 :  | 5 :  | 5 :  | 5 :  | 5 :  | 5 :  | 7 :  | 35   |     |      |
| 49  | : 31 :    | 11/18 : | 2.11 :        | 26 :    | 32 :   | 3 :                     | 3 :        | 3 :      | 3 :  | 3 :  | 3 :  | 4 :  | 5 :  | 7 :  | 7 :  | 35   |     |      |
| 1   | : 13 :    | 4/23 :  | 2.14 :        | 19 :    | 71 :   | 9 :                     | 10 :       | 10 :     | 11 : | 11 : | 11 : | 11 : | 11 : | 11 : | 11 : | 55   |     |      |
| 6   | : 13 :    | 4/23 :  | 2.15 :        | 20 :    | 50 :   | 16 :                    | 16 :       | 16 :     | 16 : | 16 : | 16 : | 16 : | 16 : | 16 : | 16 : | 80   |     |      |
| 5   | : 13 :    | 4/23 :  | 2.22 :        | 19 :    | 71 :   | 17 :                    | 17 :       | 17 :     | 17 : | 17 : | 17 : | 17 : | 17 : | 17 : | 17 : | 85   |     |      |
| 24  | : 19 :    | 6/10 :  | 2.26 :        | 24 :    | 54 :   | 18 :                    | 18 :       | 20 :     | 20 : | 20 : | 20 : | 20 : | 20 : | 20 : | 20 : | 100  |     |      |
| 23  | : 19 :    | 6/10 :  | 2.42 :        | 24 :    | 54 :   | 20 :                    | 20 :       | 20 :     | 20 : | 20 : | 20 : | 20 : | 20 : | 20 : | 20 : | 100  |     |      |
| 20  | : 19 :    | 6/9 :   | 2.51 :        | 27 :    | 52 :   | 20 :                    | 20 :       | 20 :     | 20 : | 20 : | 20 : | 20 : | 20 : | 20 : | 20 : | 100  |     |      |
| 21  | : 19 :    | 6/10 :  | 2.65 :        | 25 :    | 54 :   | 20 :                    | 20 :       | 20 :     | 20 : | 20 : | 20 : | 20 : | 20 : | 20 : | 20 : | 100  |     |      |
| 22  | : 19 :    | 6/10 :  | 2.65 :        | 25 :    | 54 :   | 20 :                    | 20 :       | 20 :     | 20 : | 20 : | 20 : | 20 : | 20 : | 20 : | 20 : | 100  |     |      |
| 4   | : 13 :    | 4/23 :  | 2.87 :        | 19 :    | 71 :   | 17 :                    | 18 :       | 19 :     | 19 : | 19 : | 19 : | 20 : | 20 : | 20 : | 20 : | 100  |     |      |
| 19  | : 19 :    | 6/9 :   | 2.90 :        | 27 :    | 52 :   | 20 :                    | 20 :       | 20 :     | 20 : | 20 : | 20 : | 20 : | 20 : | 20 : | 20 : | 100  |     |      |
| 17  | : 19 :    | 6/9 :   | 2.92 :        | 26 :    | 54 :   | 19 :                    | 19 :       | 19 :     | 19 : | 19 : | 19 : | 19 : | 19 : | 19 : | 19 : | 95   |     |      |
| 3   | : 13 :    | 4/23 :  | 2.94 :        | 19 :    | 71 :   | 20 :                    | 20 :       | 20 :     | 20 : | 20 : | 20 : | 20 : | 20 : | 20 : | 20 : | 100  |     |      |
| 18  | : 19 :    | 6/9 :   | 3.02 :        | 27 :    | 54 :   | 20 :                    | 20 :       | 20 :     | 20 : | 20 : | 20 : | 20 : | 20 : | 20 : | 20 : | 100  |     |      |
| 2   | : 13 :    | 4/23 :  | 3.30 :        | 19 :    | 71 :   | 20 :                    | 20 :       | 20 :     | 20 : | 20 : | 20 : | 20 : | 20 : | 20 : | 20 : | 100  |     |      |

\* In runs 35, 36, 37, 38, forty mice were exposed.

Runs 1 to 33, inclusive, from the first series of runs, and runs 34 to 49, inclusive, constituting the second series of runs, are plotted separately so that the two series of runs can be compared. See charts 1 and 2.

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.

Very few of the deaths occurred in the chamber, but many mice died immediately after removal from the chamber into fresh air. Most of the deaths occurred in the first 24 hr. There was a noticeable decline in deaths on the second day, while from the third to the tenth day deaths were sporadic, due in all probability to secondary infection resulting in pneumonia.

#### IV. DISCUSSION.

##### A. Concentration Data.

The agreement between the nominal and the analytical concentrations is shown in table 2. As previously explained the original analytical concentrations found for runs 1 to 16, inclusive, have been corrected by dividing them by 0.833 for reasons explained on page 4.

Table 2

Comparison of Nominal and Analytical Concentrations.

| Unlacquered chamber |               |            |           | Lacquered chamber |               |            |           |
|---------------------|---------------|------------|-----------|-------------------|---------------|------------|-----------|
| Run                 | Concentration |            | $C_a/C_n$ | Run               | Concentration |            | $C_a/C_n$ |
|                     | Nominal       | Analytical |           |                   | Nominal       | Analytical |           |
|                     | mg./l.        | mg./l.     |           |                   | mg./l.        | mg./l.     |           |
| 1                   | 2.99          | 2.14       | 0.72      | 17                | -             | 2.92       | -         |
| 2                   | 4.04          | 3.30       | 0.82      | 18                | -             | 3.02       | -         |
| 3                   | 3.36          | 2.94       | 0.88      | 19                | -             | 2.90       | -         |
| 4                   | 3.15          | 2.87       | 0.91      | 20                | -             | 2.51       | -         |
| 5                   | 2.66          | 2.22       | 0.84      | 21                | -             | 2.65       | -         |
| 6                   | 2.38          | 2.15       | 0.90      | 22                | -             | 2.65       | -         |
| 7                   | 2.06          | 1.85       | 0.90      | 23                | 2.40          | 2.42       | 1.01      |
| 8                   | 1.69          | 1.43       | 0.84      | 24                | 2.23          | 2.26       | 1.01      |
| 9                   | 1.20          | 1.02       | 0.85      | 25                | 2.02          | 1.99       | 0.99      |
| 10                  | 0.84          | 0.73       | 0.88      | 26                | 1.77          | 1.69       | 0.96      |
| 11                  | 1.47          | 1.13       | 0.77      | 27                | 1.54          | 1.49       | 0.97      |
| 12                  | 1.98          | 1.15       | 0.58      | 28                | 1.29          | 1.43       | 1.11      |
| 13                  | 2.18          | 1.42       | 0.65      | 29                | 1.00          | 0.92       | 0.92      |
| 14                  | 2.56          | 1.72       | 0.67      | 30                | 1.90          | 1.72       | 0.91      |
| 15                  | 2.73          | 1.74       | 0.64      | 31                | 1.67          | 1.40       | 0.84      |
| 16                  | 2.35          | 1.65       | 0.70      | 32                | 1.45          | 1.34       | 0.92      |
|                     |               |            |           | 33                | 1.22          | 0.84       | 0.69      |
|                     |               |            |           | 34                | 1.29          | 1.00       | 0.78      |
|                     |               |            |           | 35                | 1.42          | 1.24       | 0.87      |
|                     |               |            |           | 36                | 1.54          | 1.47       | 0.96      |
|                     |               |            |           | 37                | 1.69          | 1.62       | 0.96      |
|                     |               |            |           | 38                | 1.79          | 1.83       | 1.02      |
|                     |               |            |           | 39                | 1.93          | 1.87       | 0.97      |
|                     |               |            |           | 40                | 1.29          | 1.35       | 0.96      |
|                     |               |            |           | 41                | 1.54          | 1.62       | 1.05      |
|                     |               |            |           | 42                | 1.69          | 1.73       | 1.02      |
|                     |               |            |           | 43                | 1.79          | 1.89       | 1.06      |
|                     |               |            |           | 44                | 1.91          | 2.02       | 1.06      |
|                     |               |            |           | 45                | 1.54          | 1.57       | 1.02      |
|                     |               |            |           | 46                | 1.69          | 1.73       | 1.02      |
|                     |               |            |           | 47                | 1.79          | 1.82       | 1.02      |
|                     |               |            |           | 48                | 1.91          | 2.03       | 1.06      |
|                     |               |            |           | 49                | 2.02          | 2.11       | 1.04      |

An examination of this table leads to several conclusions. The low ratio of  $C_a/C_n$  in runs 1 to 16 is undoubtedly due to the fact that

a considerable portion of the chlorine attacked the chamber walls and was thereby removed from the atmosphere in the form of a solid chloride.  $C_n$  is not given in runs 17 to 22 since there was definite indication of turbulent flow above 9 in. on the flowmeter. These runs are not considered important in any case since the concentrations were so far above the median lethal concentration as to cause 100% deaths within the 10-day observation period, and thus could not be used in plotting the data to obtain the m.l.c. The corroboration of the  $C_n$  by the  $C_a$  in runs 23 to 29 shows that the lacquer coating gave complete protection to the chamber walls. After run 30 there was some visual evidence of corrosion in the chamber and this may partly explain the irregular ratio in runs 30-33. The chamber was relacquered before run 34 and it will be noted that there was very good agreement between  $C_a$  and  $C_n$  in most of the remaining runs in the table.

Since the analytical concentrations checked the nominal concentrations in the runs where it was known there was no corrosion of the chamber the former were used for the computation of the median lethal concentrations.

Sources of experimental error are tabulated below:

|                                                                                                                                                                                                                                                                                                                                             | <u>Per cent error</u> |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| 1. <u>Chemical and Physical.</u>                                                                                                                                                                                                                                                                                                            |                       |
| a. Purity of compound, 99.7%.                                                                                                                                                                                                                                                                                                               | 0.03                  |
| b. Analytical concentration, $C_a = \frac{m}{v}$<br>$m$ = total milligrams found, depending on<br>titrating solution.<br>ml. $AgNO_3$ used near m.l.c. =<br>$4 \pm 0.02$ ml. <span style="float: right;">0.5</span><br>$v$ = volume of sample, measured on corrected<br>wet meter = $10 \pm 0.01$ l. <span style="float: right;">0.1</span> |                       |
| c. Nominal concentration, $C_n = R/Q$<br>$R$ = weight-loss/time, measured on a flowmeter,<br>accuracy about <span style="float: right;">4.0</span><br>$Q$ = air flow through chamber. <span style="float: right;">4.0</span>                                                                                                                |                       |
| Total possible known analytical and nominal errors<br>involved, including purity: about                                                                                                                                                                                                                                                     | 8.6                   |

Per cent error

d. Accuracy of plot.

|                                               |         |
|-----------------------------------------------|---------|
| 1 division = 0.02 mg./l.; at m.l.c.           | 1.2     |
| 1 division = ca. 1% between 25 and 75% deaths | ca. 1.0 |

2. Biologic.

a. Animals.

|                                                    |                |
|----------------------------------------------------|----------------|
| Lot 13, runs 1-11, normal 10-day death rate, 0.0%  | Indeterminable |
| Lot 15, runs 12-16, normal 10-day death rate, 14%  | "              |
| Lot 19, runs 17-27, normal 10-day death rate, 0.0% | "              |
| Lot 18, runs 28-29, normal 10-day death rate, 1.7% | "              |
| Lot 20, runs 30-33, normal 10-day death rate, 2.9% | "              |
| Lot 30, runs 34-44, normal 10-day death rate, 0.0% | "              |
| Lot 31, runs 45-49, normal 10-day death rate, 0.0% | "              |

b. Physiologic variations due to age, sex, previous nutritional history, etc. Probably much more significant in runs 1-33 than in runs 34-49. Indeterminable, but large in some runs.

B. Median Lethal Concentration.

In view of the fact that the m.l.c. of chlorine was requested as a standard for comparison purposes, the work herein described was done with more than the usual care. Almost five times the customary number of runs were made and all readings were checked twice.

It should be noted, however, that although the errors involved in the chemical and mechanical procedures have been reduced to a minimum, various sources of physiologic error remain. An example of this is found in the fact that the mice used at Edgewood Arsenal are purchased from dealers and that mice from 7 different purchase lots were used in the work on chlorine here reported. Even if the mice used in the several runs were

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all of the same age and in the same sex ratio, it is probable that animals of different strains and of different nutritional histories would vary considerably in their resistance to the effects of gassing. In this particular instance, moreover, a marked variation in resistance might well be expected, since the "normal" 10-day death rates in the seven purchase lots were 0.0, 14.0, 1.7, 0.0, 2.9, 0.0 and 0.0%, respectively.

As stated previously the runs recorded in this report really represent two separate and distinct investigations. The first such study covers runs 1 to 33, inclusive, and the second covers runs 34 to 49, inclusive. In the first series of runs five different lots of mice were used having 10-day death rates ranging from 0.0 to 14%. Little was known of the history of these mice, their weights, sex ratio, age, or pathological variation. In the second series only two lots of mice were used and the 10-day death rates for both lots were 0.0%. Both lots were purchased under carefully drawn specifications and are believed to have been substantially free from pathological variations. It would be expected that the m.l.c. for the two investigations would differ somewhat and that the m.l.c. for the second lot would be somewhat higher than that for the first. This is actually the case. The results of the two series of runs have been plotted separately and separate conclusions have been drawn.

In order that a valid comparison may be drawn between the work of the Chicago laboratory of the N.D.R.C. and the Edgewood Laboratory of the Medical Research Division, a third investigation has been conducted on chlorine and will be reported in a separate report (E.A.T.R. 373). An arrangement has been made with the N.D.R.C. and with the Carworth Farms, whereby both the Chicago laboratory and the Edgewood laboratory will be supplied with all male mice of the CF-1 strain for future mouse toxicity work.

#### V. CONCLUSIONS.

1. When the mice used were selected at random from miscellaneous lots, the m.l.c. of chlorine for an exposure period of 10 min. and an observation period of 10 days was  $1.52 \pm$  an average deviation (for 20 runs of 20 mice each) of 0.19 mg./l.

2. When mice of carefully selected strain having a daily death rate for 10 days of 0.0% were used, the m.l.c. of chlorine for an exposure period of 10 min. and an observation period of 10 days was  $1.73 \pm$  an average deviation for 20 runs of 0.24 mg./l.

#### VI RECOMMENDATION.

None, since further work is in progress and will be reported in E.A.T.R. 373.

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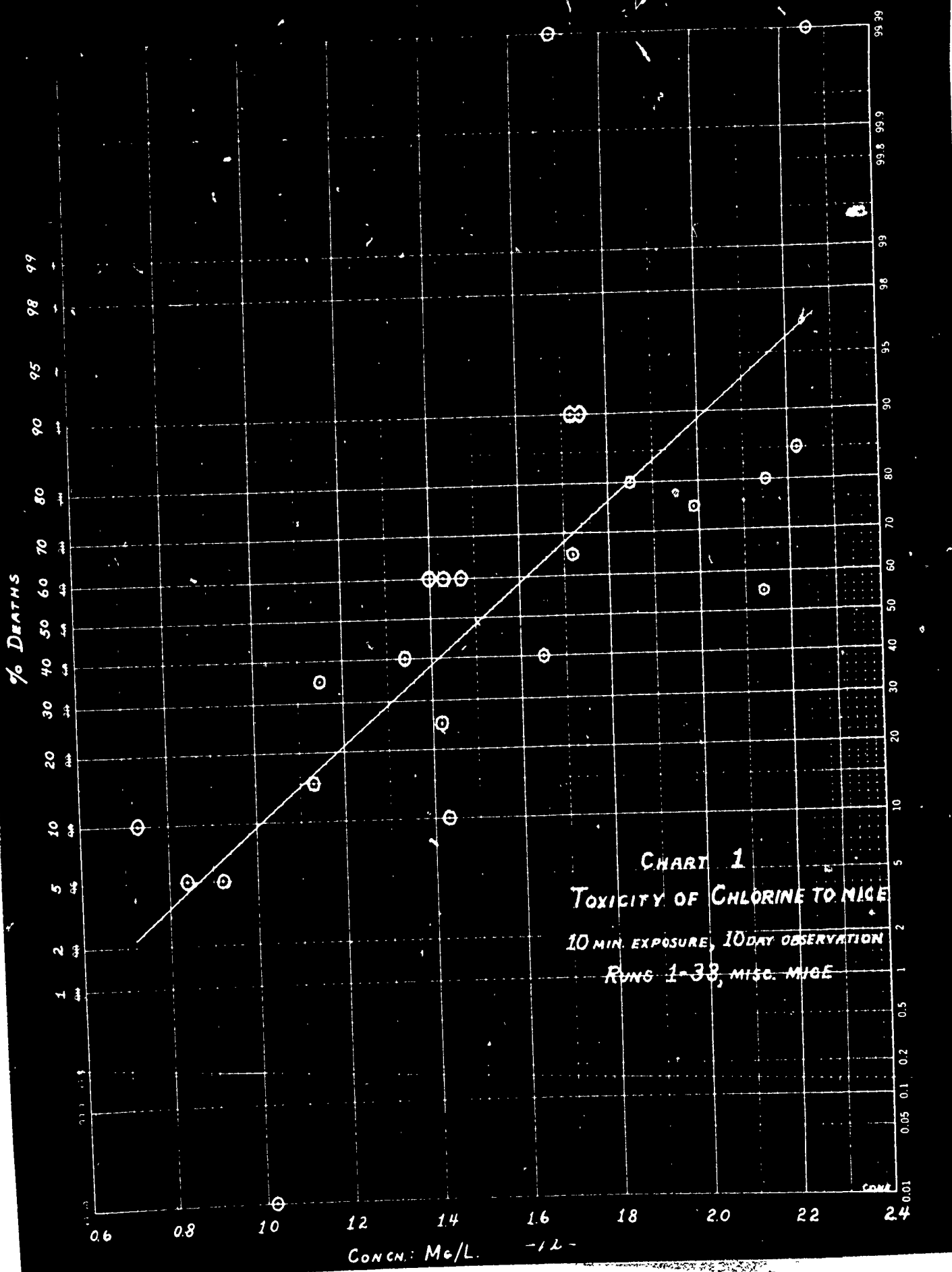
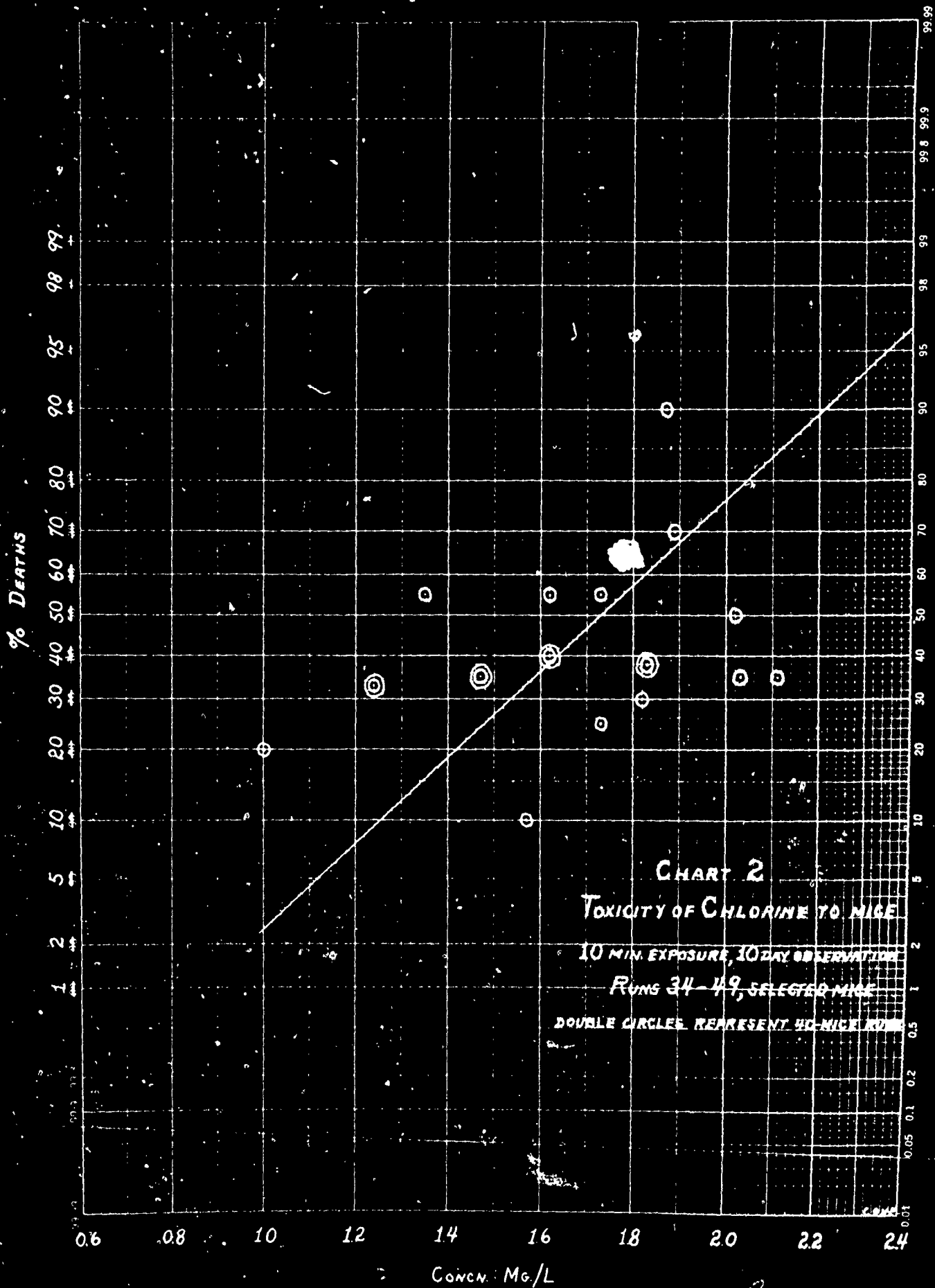


CHART 1  
 TOXICITY OF CHLORINE TO MICE  
 10 MIN. EXPOSURE, 10 DAY OBSERVATION  
 RUNS 1-38, MISC. MICE

CONCN: Mc/L.



CONCN. MG./L



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 E.A.T.R. 351.

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