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Report No. 205

COMPARATIVE STUDY OF GB INHALATION TOXICITY ON MICE, RATS, GUINEA PIGS, CATS, DOGS AND MONKEYS WITH EXPOSURE TIMES BETWEEN ONE SECOND AND SEVERAL MINUTES

by

H. J. Trurnit E. J. Esposito

P. D. Bales P. Horowitz

with the technical assistance of

[redacted]

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August 1953

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CHEMICAL CORPS
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CMLRE-ML-52

Medical Laboratories Research Report No. 205

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Comparative Study of GB Inhalation Toxicity on Mice, Rats, Guinea Pigs, Cats, Dogs and Monkeys with Exposure Times Between One Second and Several Minutes

ABSTRACT

OBJECT.

The purpose of this study was to obtain comparable LCt_{50} figures with a variety of species for high concentrations of and very short exposures to GB vapor.

CONCLUSIONS.

1. The LCt_{50} of GB vapor for mice, rats, guinea pigs, cats, dogs and Rhesus monkeys decrease with decreasing exposure time.

2. This relationship, except for monkeys, may be expressed by the general formula $LCt_{50} = k \times t^n$. The values for k are: 169, 117, 100, 54, 79 for the species as listed above. The n values are 0.34, 0.23, 0.27, 0.27 and 0.42.

3. The data on monkeys show a constant LCt_{50} of 25-30 mg. min./m.^{3} down to 10 second exposures and then drop down to 5 mg.min./m.^{3} for 2 second exposures.

4. A computation of LD_{50} from these data shows that less agent is required to kill with a two second exposure than is necessary to kill with intravenous injection of GB.

5. An extrapolation to man indicates that an LCt_{50} of 3-5 mg.min./m.^{3} may be expected if a sufficient amount of agent is inhaled within one single breath of one liter volume. The corresponding concentration is 100 mg./m.^{3}.

6. The very short incapacitation time connected with LCt_{50} at high concentrations seems to make the self administration of first aid impossible.

RECOMMENDATIONS.

None.

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Medical Laboratories Research Report No. 205

Comparative Study of GB Inhalation Toxicity on Mice, Rats, Guinea Pigs, Cats, Dogs and Monkeys with Exposure Times Between One Second and Several Minutes

I. INTRODUCTION.

A. Object.

The purpose of this study was to obtain comparable LCt_{50} figures with a variety of species for high concentrations of and very short exposures to GB vapor. In order to connect the results with those of other investigators and to obtain, if possible, a more generalized insight into the toxicity mechanism, longer exposures up to several minutes were made as well.

B. Authority.

Cml C, Project Spec. 4-08-02-007, Toxicology of CW Agents Dispersed as Vapors. (SOA 04847).

II. HISTORICAL.

The literature studed (2-9) was found to contain limited information on the LCt_{50} of inhaled GB vapor for short exposure times. The earliest report obtainable (1) contains pharmacological data on Tabun, Sarin and Soman, (GA, GB and GD). The data in this report appear rather sketchy since test method, exposure time and type of gassing chamber are not mentioned - nevertheless, the toxicities of these compounds as estimated at the Military Academy of Berlin indicated that at greater concentrations the LCt_{50} was smaller.

The survey of available data reveals two general trends. First, the LCt_{50} data are higher for smaller experimental species than for larger ones. Second, the LCt_{50} decreases towards shorter exposure times more for smaller animals than it does for larger animals. For mice, rats and guinea pigs the 10 minute value of the LCt_{50} is 3 to 4 times that of the 10 second value. For cats it is about twice as much and for sheep and monkeys there is no difference in the LCt_{50} for 5 seconds and 1 minute exposure times. Pigeons are more sensitive to GB vapor than the other laboratory animals if the body weight is taken as a criterion. The LCt_{50} in this case is about 4 times as high with 3 minutes' exposure than it is for 3 seconds' exposure.

It has been reported (2) that maximum miosis and strong dyspnea in man after a 5 to 10 minutes exposure is obtained with a concentration of 0.5 to 1.0 mg. GB per cubic meter.

III. EXPERIMENTAL.

A. General.

For exposure times in the range of several seconds, the usual gas chambers and sampling techniques are not suitable.

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There a cage with animals is pushed into a much larger chamber filled with toxic vapor and the fresh air contained in the cage is added to and mixed with the chamber mixture. Because the complete removal of this fresh air takes at least several seconds, this procedure introduces an error of varying and unknown magnitude. Therefore, a different type of chamber had to be used.

In order to take vapor samples from the chamber during the exposure, this sampling has to be done within seconds. The chamber concentrations needed for lethal Ct_{50} values with these short exposure times are such that at least one-half liter of chamber air has to be sampled for a colorimetric analysis. It is quite impossible to pull one-half liter of gas in 1 or 2 seconds through a liquid sampler without considerable loss of agent; therefore, a more rapid sampling technique had to be employed.

The dimensions of the new chamber, the necessary chamber flow rate and the need for concentrations up to 2 g. per $m.^3$ made it necessary to design a special disperser with a vaporization rate of 3 ml. GB per minute. Furthermore, this disperser had to be unbreakable, safe and simple in handling.

B. Chamber.*

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*A more detailed description of the chamber will be given in a report by P. Bales.

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D. Agent.

Several 1 liter batches of liquid GB were used. The purity, based on P and F determinations ranged from 97-99%, the acidity from 10-15 mg. H-ions per 100 g. agent. Source of agent and analytical data: Chemical Division, Cml C Chemical and Radiological Laboratories, Army Chemical Center, Md.

E. Sampling.*

In order to take gas samples from the chamber <u>during</u> the exposures, a rapid sampling method is essential. Hand operated 1 liter metal piston pumps are used to draw, within one to two seconds, one liter of gas through 3 grams of pre-treated silica gel. This method was checked for a concentration range of 100-2000 mg./m.^{3} against the tetralin sampling technique and found to be in close enough agreement to justify its use.

F. Analysis.

The GB was desorbed from the silica gel with isopropyl alcohol and determined colorimetrically by the dianisidine method under standardized conditions.

G. Animals.

A total of 390 mice, 324 rats, 260 guinea pigs, 40 cats, 21 dogs and 38 monkeys were used for this study. Ten mice or 6 rats or 4 guinea pigs were always exposed simultaneously. Cats, dogs, and Rhesus monkeys were exposed singly. Weights and sex of the animals were recorded before each experiment. The overall average weights of the different species in grams were as follows: Mice: 31 (25-37); rats: 293 (195-367); guinea pigs: 386 (295-527); cats: 2830 (1910-4400); dogs: 5884 (3860-7900); monkeys: 3167 (2270-4400). Prior to exposure, mice, rats and guinea pigs were kept at least one week in the laboratory for adaptation and observation of possible epidemic diseases. Symptoms, during and after exposure, were recorded in the case of cats, dogs and monkeys. The survival time was recorded to the nearest minute. No physiological tests or measurements were made, the only criterion being death or survival. Except for the monkeys, whose arms and legs were tied together, the animals were free to move in their cages during and after exposure. After 24 hours all surviving animals (except dogs and monkeys) were sacrificed and together with the lethal cases subjected to a gross pathological inspection (See Appendix II).

H. Procedure.

A certain vapor concentration was established and maintained constant. Then between 4 and 8 cages with several animals per cage (for the smaller species) or between 4 and 8 single animals of the larger species were exposed to this concentration for various lengths of time. The time range was aimed at covering the range of partial response only (when more than one animal was in the chamber) or at obtaining several deaths as well as no death responses within that exposure time range. The actual mechanism of exposure is as follows: By throwing the valve handle, the toxic vapor was brought to the animals as a column with a "sudden"

*A separate report will give the full details of the sampling and analytical procedures.

front. The end of the exposure was also marked as a sudden front of fresh air arriving at the cage. In order to protect the animals from obtaining agent before and after the actual exposure (leak of valve or desorption from the walls), the time during which the animals stayed in the exposure compartment before and after exposure was cut down in most cases to several seconds, especially with high concentrations. (See Appendix IV).

The procedure during this study was approximately this: a. Filling and marking the silica gel cartridges. b. Weighing and distributing the animals in labelled cages. c. Preheating and adjusting the disperser for a particular concentration. d. Beginning the exposures at about 1000 hours.

The operating time of the disperser was kept as short as possible in order to save agent. The delaying factor, however, was the necessity to watch the symptoms after each exposure in order to estimate the probable result and thereby the next exposure time. These observation periods varied for different species. In the beginning, it was tried to save agent by cutting down the disperser flow rate between exposures. This gave irregular analytical results due to the disturbance of the thermal equilibrium in the disperser system. This procedure was soon given up. Shortly before or after noon time, the animals that had died during the morning's run and those that had survived from the previous day were used for a gross pathological examination. Few animals died more than 2 hours after exposure.

I. Results.

All experimental data are presented in Appendix I. The results are plotted in Figures 5-10. The abscissae show time in seconds on a logarithmic scale covering about 16 minutes. The ordinates show the product Ct in mg. x min. per $m.^3$ on a logarithmic scale. In Figures 5-7 each circle represents one cage. The number of deaths occurring in that cage during 24 hours after exposure is inserted in the circle. In Figures 8-10, each circle represents one single animal. Full circles indicate deaths within 24 hours, open circles survival. The straight lines in Figures 5-9 are statistical LCt_{50} regressions. They are based on a Bliss analysis (in Figures 5-7) and on a "discriminant functions" analysis (in Figures 8 and 9)*. The equation of the straight line in all cases is of the type

$$LC \times t_{50} = kt^n$$

The k and n values are given in Figure 11.

The straight lines seem to fit the results reasonably well. The data for monkeys were not analyzed statistically because they do not fit a linear expression in either a bilogarithmic or semi-logarithmic plot.

Table 1 correlates for such exposure group the survival time and the exposure time range. With mice and rats, death occurs on the average later with increasing exposure times. For the four other species this does not seem to be the case. The corresponding data for cats, dogs and monkeys are plotted in Figure 12. With mice, rats and guinea pigs most deaths occur within the first fifteen minutes; with dogs, within the first two hours; while all the deaths in monkeys occurred within fourteen minutes, the shortest survival time there being one minute. The longest average survival time is found with cats.

*See Appendix III.

The experimental data for cats, dogs and monkeys (Appendix I) contain tabulations of the occurrence of certain symptoms. These data as such are correct but they are not complete. The observation of symptoms was only done as far as time was available. Minor symptoms may not have been noted if severe symptoms appeared first. Some animals had severe convulsions without prior minor symptoms. Despite the fragmentary character of these data, they are included in the report as a basis for further studies.

The data show, for example, that without exception all cats that died had severe convulsions first, but not all monkeys and dogs. Dogs and monkeys without severe convulsions before death collapsed and showed flaccid paralysis in many cases and fine muscular fibrillations all over the body. With cats and monkeys a few animals that had severe convulsions survived but all dogs with this symptom died.

Cyanosis was easiest to detect in monkeys, less so in dogs, and still less in cats. Myosis was easiest to observe in cats. The term respiratory difficulty covers symptoms ranging from slight coughing, retching and panting to deep forced respiration and diaphragmatical spasm.

In many cases animals not showing any more signs of respiration or pulse and being considered dead showed muscular fibrillations or twitchings of the ears (monkeys) for many minutes after "death" or made, unexpectedly, one more deep gasp. One guinea pig which was considered dead for about fifteen minutes and therefore not watched carefully was found to be completely recovered ten minutes later ! One monkey (serial #165) was deeply unconscious when taken out of the chamber several seconds after a two second exposure. He made several deep gasps, showed muscular twitching all over his body and died after about one minute. This animal obtained approximately two lethal doses but certainly not more than three lethal doses as judged by the combined data in that range (see Figure 6).

IV. DISCUSSION.

Before starting this study the general impression from previous work was that only the smaller animals (mice, rats, guinea pigs and cats) showed a decreasing LCt_{50} with decreasing exposure time. The results of the present work, however, indicate that this decrease holds for larger animals too, at least in the short exposure time range. In the range between two seconds and three minutes the correlation between logarithm of exposure time and logarithm of LCt_{50} seems to be linear for rats, guinea pigs and cats. For mice and dogs this linear relationship seems to hold up to one minute. Beyond one minute the data obtained for mice are lower than indicated by the exponential formula. And for dogs, where no data above one minute were obtained, it is known from previous work that the LCt_{50} for ten minutes is 120 mg. min./m.^{3} (10). From the regression obtained in this work, it would come out to be 208.

The results from the experiments with monkeys seem to indicate a fairly constant LCt_{50} of approximately 25 mg. min./m.^{3} for exposure times down to ten seconds.* Then a sharp drop occurs to an LCt_{50} in the neighborhood of 5 mg. min./m.^{3} for exposure times from one to two seconds. Comparing the data of all six

*See Appendix V.

species, one might hazard the guess that with increasing overall sensitivity of the species towards GB the transition between an approximately constant LCt_{50} and declining LCt_{50} shifts toward shorter exposure times.

The data for cats and dogs show less spread than those for mice, rats and guinea pigs. This may be partly connected with the facts pointed out in Appendix II. The smaller species were not as healthy groups as were the cats and dogs. The irregularities (overlapping) with monkeys in the exposure time range from one to ten seconds cannot be explained. The tying together of arms and legs and a possible inhibitory effect of this on the respiration cannot explain this difference below and above ten seconds exposure time.

An attempt has been made to calculate from these data the LA_{50}'s* and relate these figures together with injection toxicity data to the average weight of the animals used. The LCt_{50}'s for two-second and for ten-minute exposures with mice, rats, cats and dogs have been multiplied by the corresponding respiratory minute volumes as calculated by Guyton's formula (11), using the factors given in the last column of Table 2 of Guyton's paper. For cats and dogs, the factor 2.1 has been employed. Table 2 shows the full data employed in obtaining the LA_{50}'s.

The last two columns in Table 2 contain "corrected data". These were obtained by deducting a certain percentage "loss" of agent in the upper respiratory tract including the trachea and bronchi so that these corrected LA_{50}'s represent the amount of agent reaching the alveolar part of the lung.**

Plotting these data with the LA_{50}'s implies the tentative assumption that only the fraction of agent that reaches the alveolar part of the lung participates in the sequence of symptoms leading to death. This assumption seems improbable. Nevertheless, Figure 11 indicates that the slope for these corrected data approaches much more closely the slope for the intravenous injection data than does the slope for the uncorrected LA_{50}'s. The data for intravenous injection have been added as a more reliable backbone to the diagram with which the inhalation data may be compared.*** Figures for pigs, goats and roaches are

* A stands for amount of agent per animal, as D stands for amount per kg.

** This correction is based on a calculation of the adsorption loss on the walls of the upper respiratory tract and trachea and will be presented in a separate report by Trurnit.

*** Unreported data obtained by the Pharmacology Branch indicate that over an injection interval of up to 4 hours the LD_{50} of GB is independent of the injection time. The injection time of those experiments on which the injection LA_{50}'s in Figure 13 are based was always below 5 seconds.

included because they show that over a weight range from one gram to fifty thousand grams the logarithm of the LA_{50} for GB is very nearly a linear function of the logarithm of the weight.*

The comparison between inhalation and injection toxicity in Figure 10 shows that aside from the difference in slope, ten minute exposures require more agent than injection experiments. However, two second exposures require less agent than injection experiments. This difference for two second exposures becomes more pronounced with increasing weight of the species.

A very crude extrapolation to man has been made by connecting the points for mice and dogs in each case by a straight line and extending this line to its crossing point with the 70 kg. ordinate. It appears that the ten minute exposures (corrected and uncorrected) indicate LA_{50}'s between 500 and 1000 micrograms for man and the two second exposures indicate LA_{50}'s near 100 micrograms. Inhalation experiments with goats and horses are planned which will permit an interpolation rather than an extrapolation for man.

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3. ML Technical Information Reports: Toxicity by Total Exposure, 26 January 1948.

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5. Porton Memo No. 39, 2 August 1950.

6. Porton Report No. 2756, 1 February 1951.

* This relationship has the quantitative formulation (as derived from the diagram) $LA_{50} = 0.22\,W^{0.718}$ where D is given in micrograms and the weight W in grams. The exponent of W is nearly the same as the exponent 0.734, which governs the relation of body weight to metabolic rate in higher animals. This might be more than a coincidence and could indicate that the blocking of one or several links in the metabolic reaction chain is the actual cause of death after GB poisoning. Cholinesterase is not known to be a link in this chain. So we must assume that the G-agents act simultaneously and independently on the cholinesterase system and on some system belonging to the metabolic chain, or that cholinesterase inhibition has an unknown generalized effect on the overall metabolism in addition to the known effect on the respiratory center.

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8. Annual Report on Special Weapons Research, DRB Canada, Report No. DR 23, 31 May 1951.

9. Porton Report No. 19, 31 March 1952.

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Table 1

Number of Animals of Each Concentration Group Dying in the 1st., 2nd., 3rd., etc. Minute*

Serial Numbers	Range of Exposure Time in Seconds	Minutes After Beginning of Exposure: 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Deaths After 15 Min.	Total Number of Deaths in Group
MICE																		
53-59	5-14	12	12	3							1							28/70
242-249	6-18	16	9	1							1						2	29/80
47-52	7-20	15	8	2					1	2	1						1	30/60
41-46	13-30	9	21		3												1	34/60
35-40	25-60	9	18															27/60
29-34	165-285		1		3	15	1	8	1									29/60
RATS																		
66-71	4-7		2	9	5	3								1			1	21/36
60-65	4-8		1	5	8	5	1	1					1					22/36
24-28	3-12		1	5	1	5	2							1			1	16/30
19-23	5-20		1	1	5				3									10/30
7-12	15-60			9	4		3			2							2	20/36
2-6	50-100			2	1	1		1	2								1	8/30
13-18	180-420						1		2	4	1	4		2			2	16/36
GUINEA PIGS																		
93-99	6-8				2	1	2	4	1								1	11/28
106-110	6-8			2	1			1	1	1		1		2				9/20
79-85	17-25					6	2	2								1	2	13/28
224-228	35-90		1	4	4	2	1		1				1				3	17/20
251-257	60-130				1	3	2	1	1				1	1			3	13/28
72-78	110-195					2		4	3	3	2	1		1			4	20/28

* Certain groups were excluded from this table because the chamber concentration varied too much or the results were not comparable for other reasons.

TABLE 2

Species	Average Weight (W) g.	$(W)^{3/4}$	Factor	Minute Volume ml.	LCt_{50} 10 min. exp. mg.min./m.^{3}	LCt_{50} 2 sec. exp. mg.min./m.^{3}	LA_{50} 10 min. exp. mmg.	LA_{50} 2 sec. exp. mmg.	Correction Factor %	Corrected LA_{50} 10 min. exp. mmg.	Corrected LA_{50} 2 sec. exp. mmg.	LA_{50}*** I.V. injection mmg.
Mouse	31	12.9	2.54	40*	300**	53	12.0	2.1	14	1.7	0.3	3.1
Rat	293	70.9	2.10	149	220**	54	32.8	8.0	40	13.1	3.2	13.2
Cat	2830	390.	2.10	820	103	22	84.	18.0	56	47.	10.0	51.
Dog	5884	669.	2.10	1400	120**	19	168.	168.	63	106.	16.8	112.

* For mice the calculated minute volume is 33 ml. Experimentally Guyton found higher figures (24.5 instead of 19.9 as calculated for his 20 g. mice). 40 ml. instead of 33 has therefore been adopted here.

** The LCt_{50} values for 10 minute exposures of mice, rats and dogs have been taken from (12).

*** The data are calculated from the table in (12).

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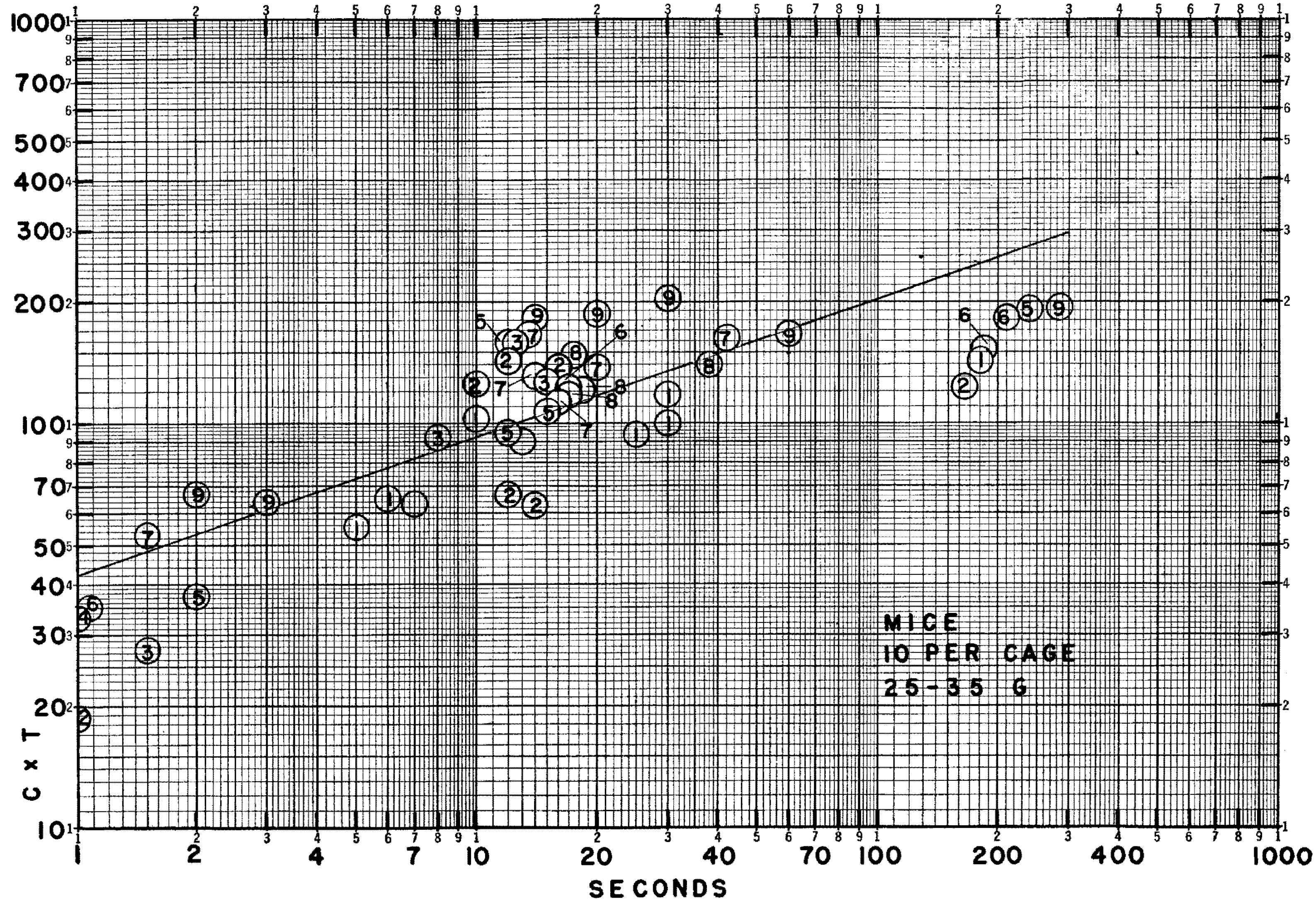
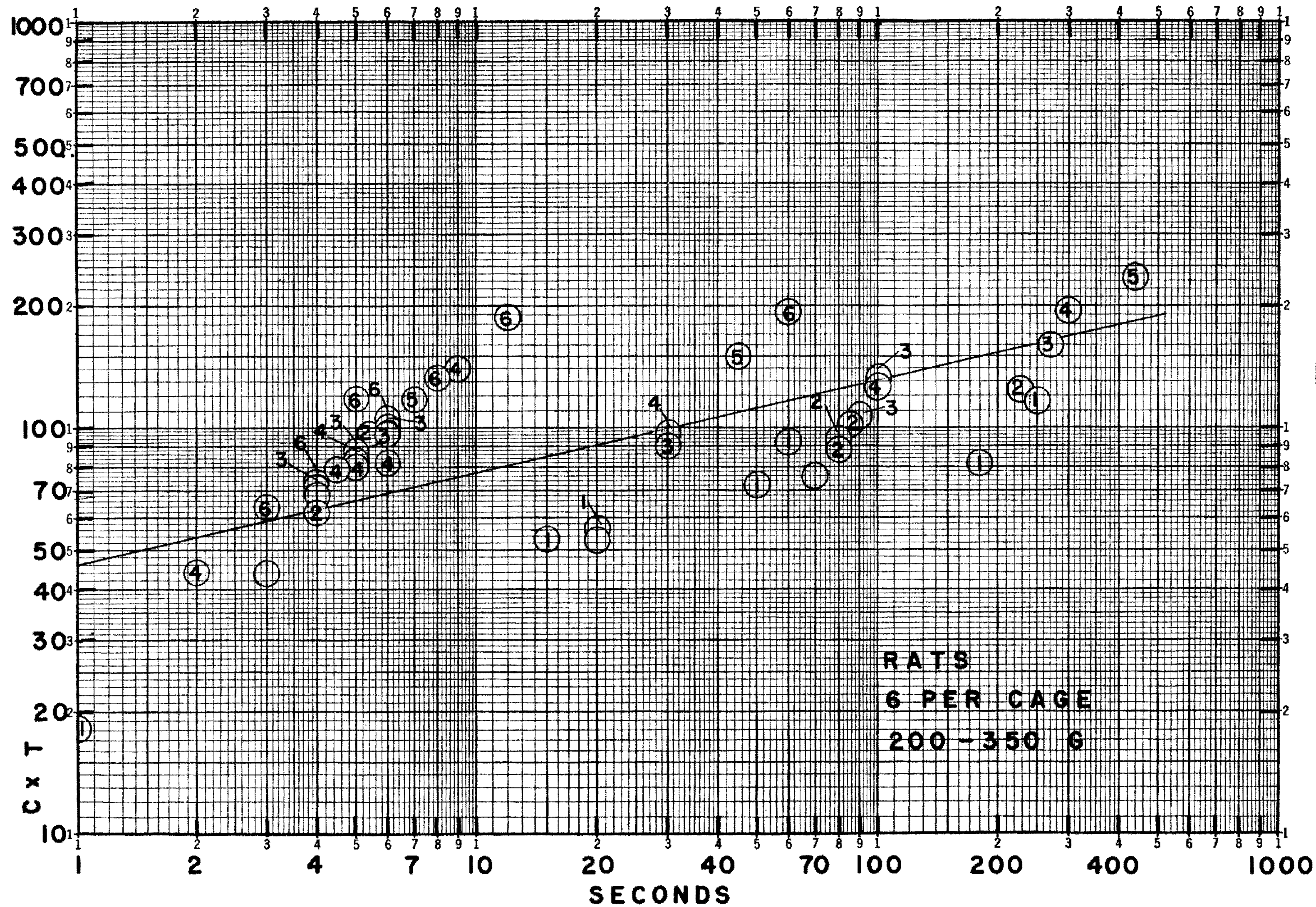


Figure 5


RATS
6 PER CAGE
200 - 350 G
C x T
SECONDS
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700
500
400
300
200
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4
7
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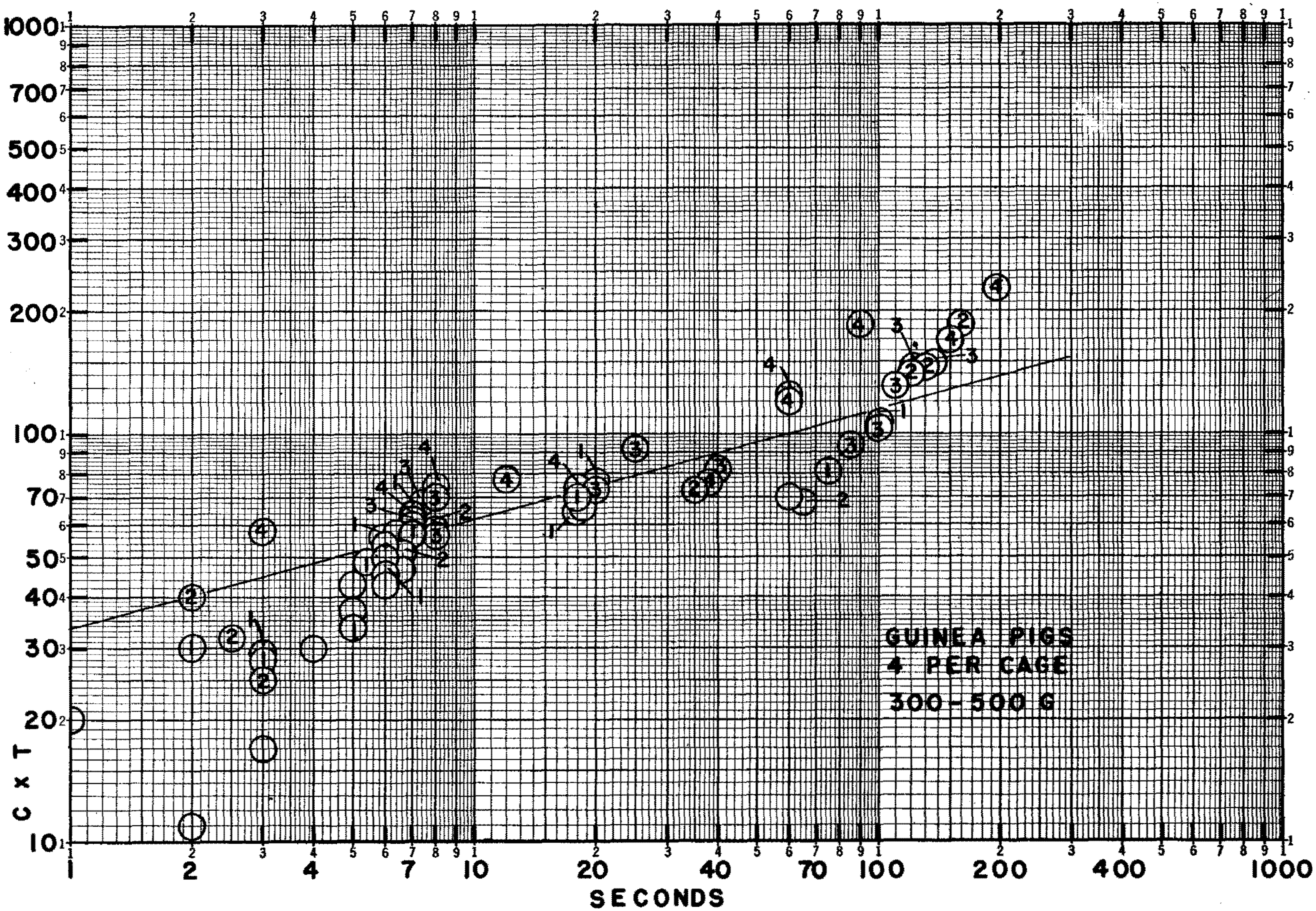
20
40
70
100
200
400
1000

Figure 6


GUINEA PIGS
4 PER CAGE
300-500 G
C x T
SECONDS
1000
700
500
400
300
200
100
70
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40
70
100
200
400
1000

Figure 7

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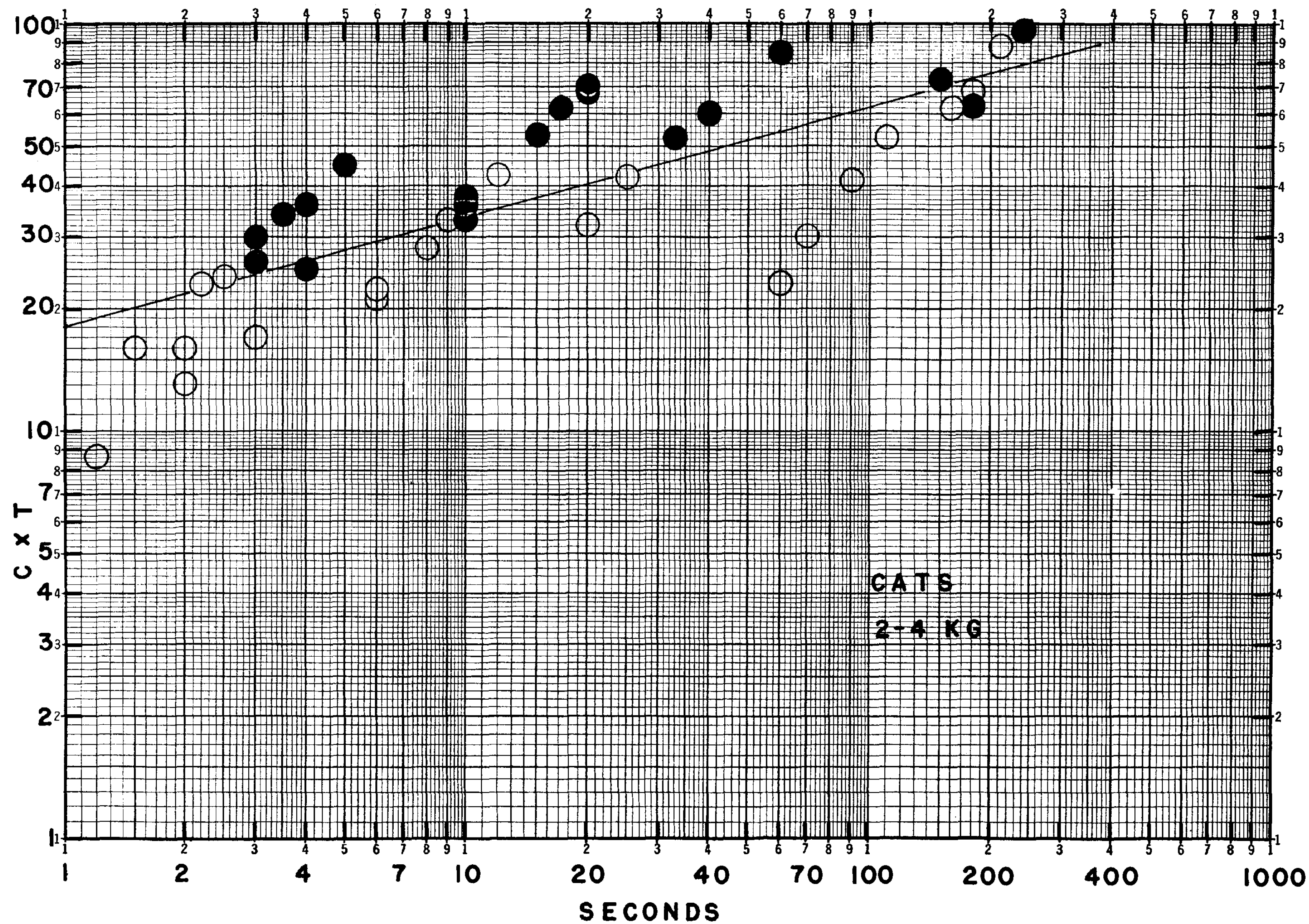

Figure 8

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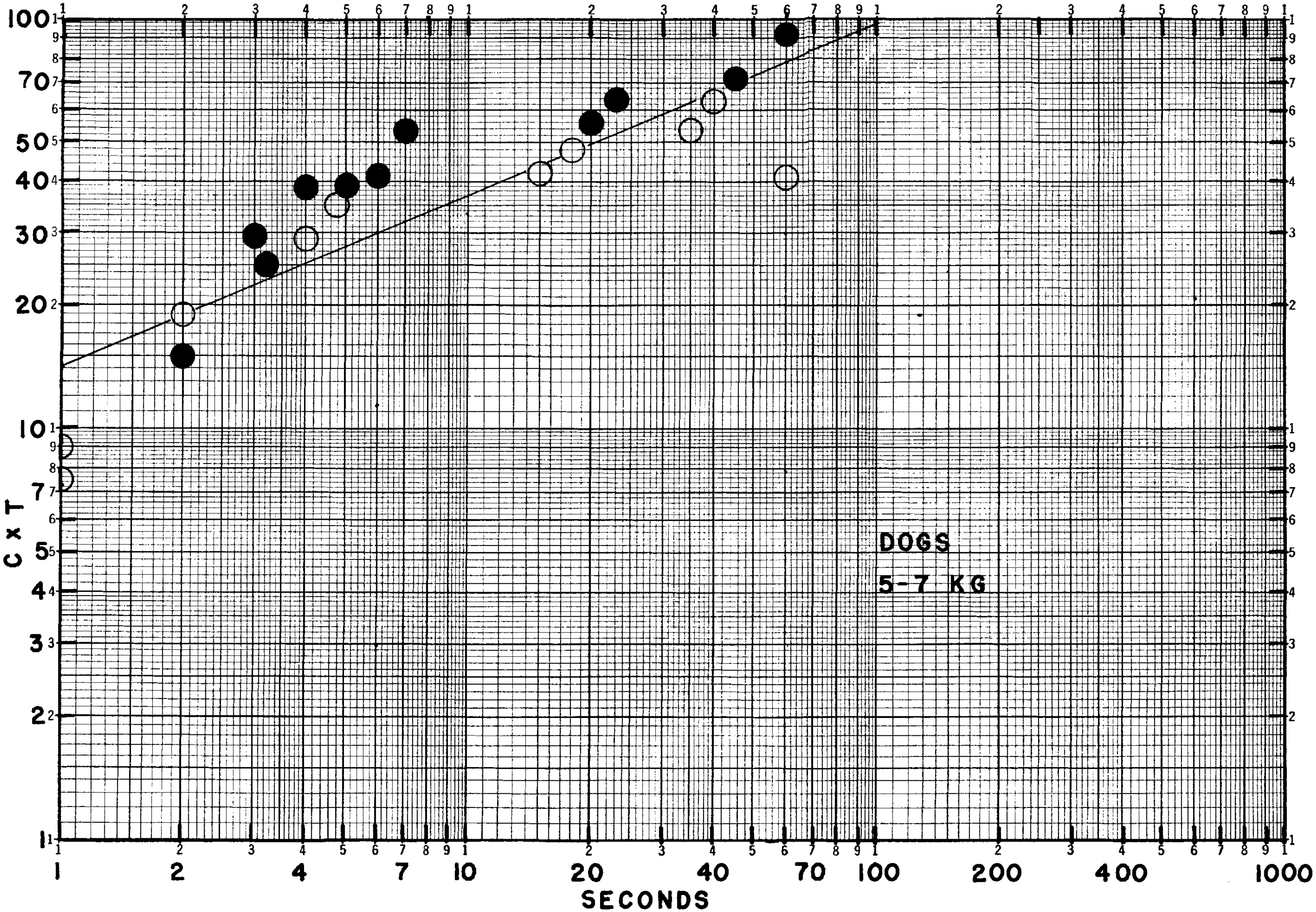

Figure 9

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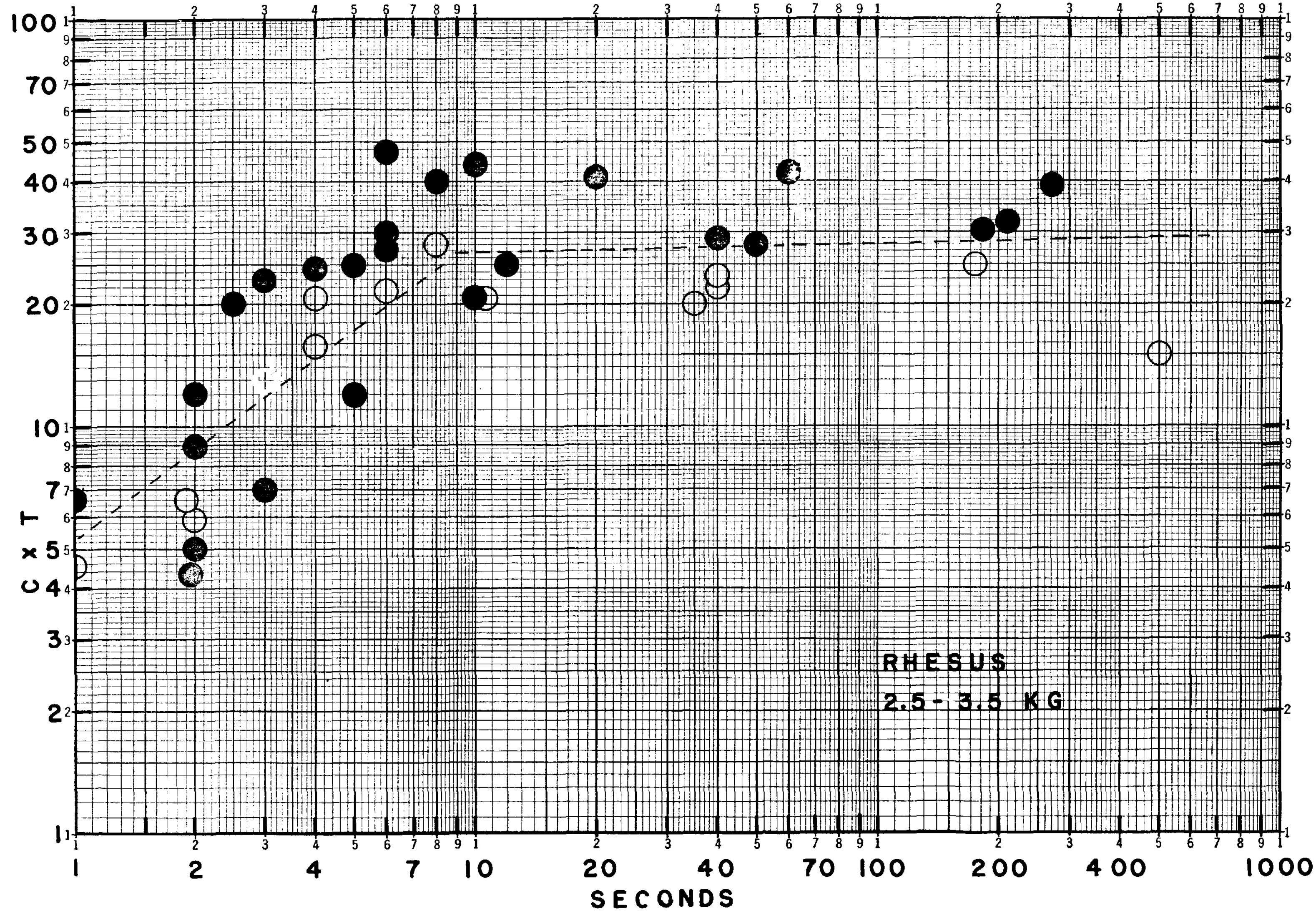

100
70
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C x T
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SECONDS
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2.5 - 3.5 KG

Figure 10

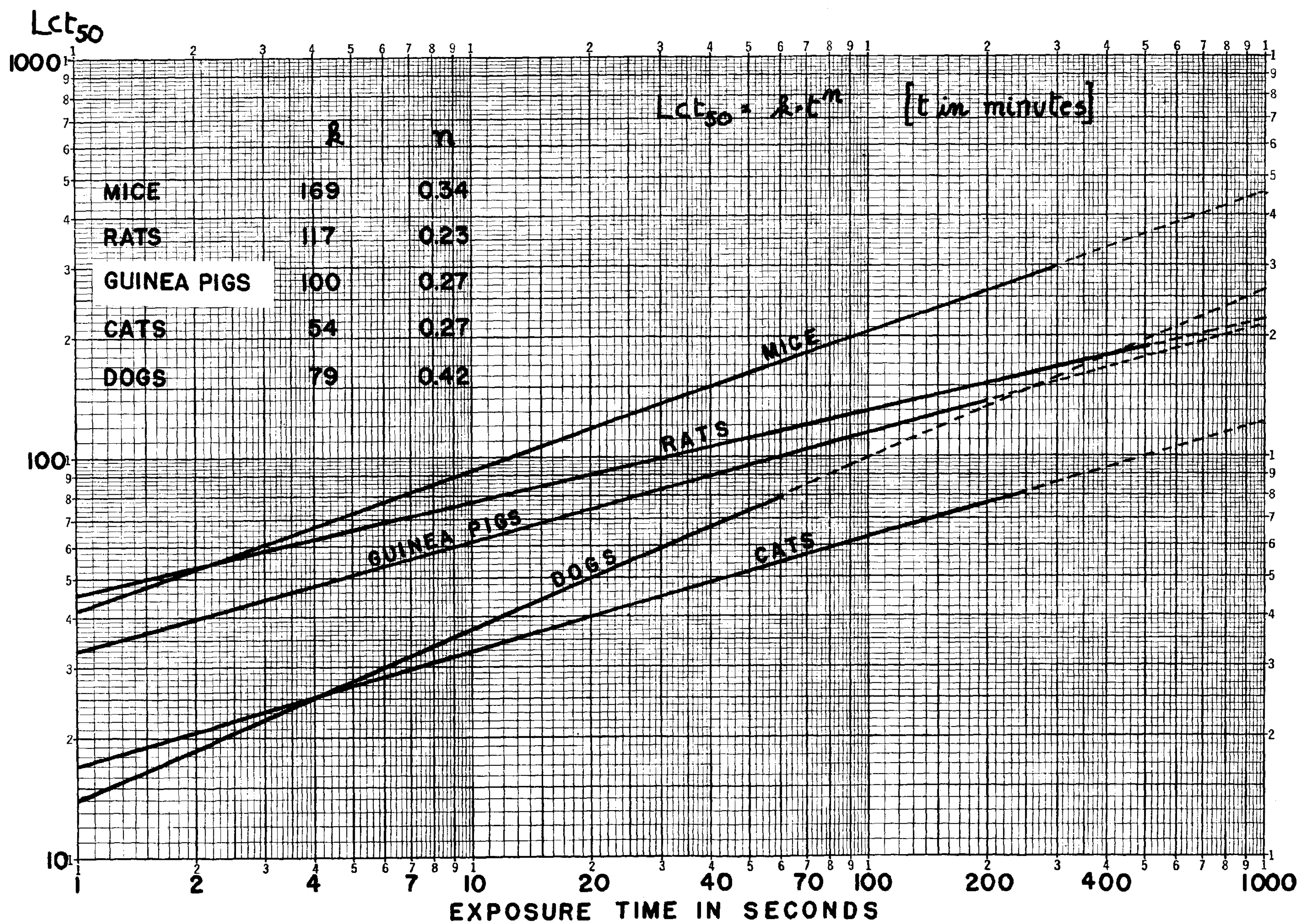

Figure 11

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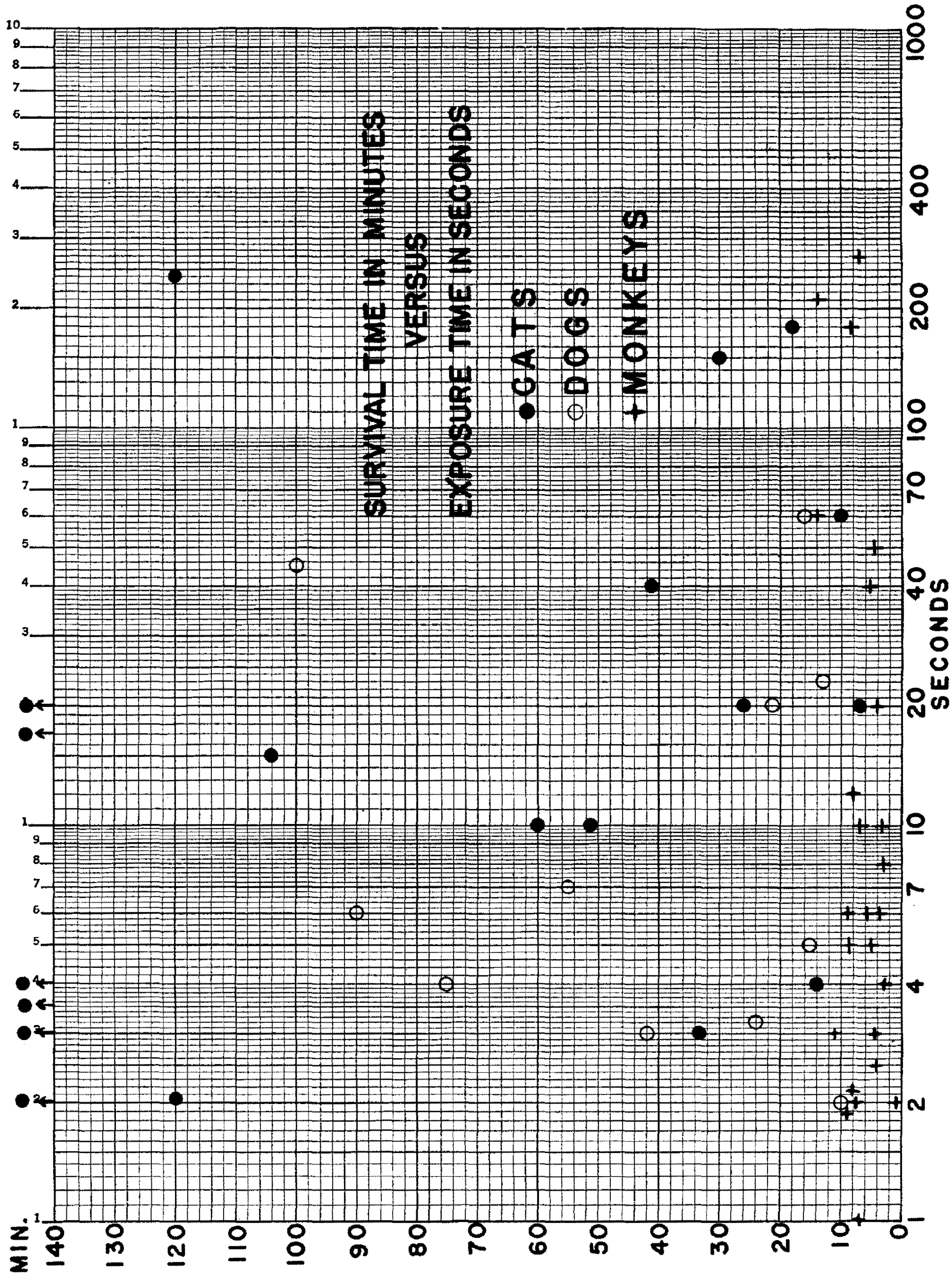
SURVIVAL TIME IN MINUTES
VERSUS
EXPOSURE TIME IN SECONDS
CATS
DOGS
MONKEYS
SECONDS
MIN.
140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
1 2 4 7 10 20 40 70 100 200 400 1000

Figure 12

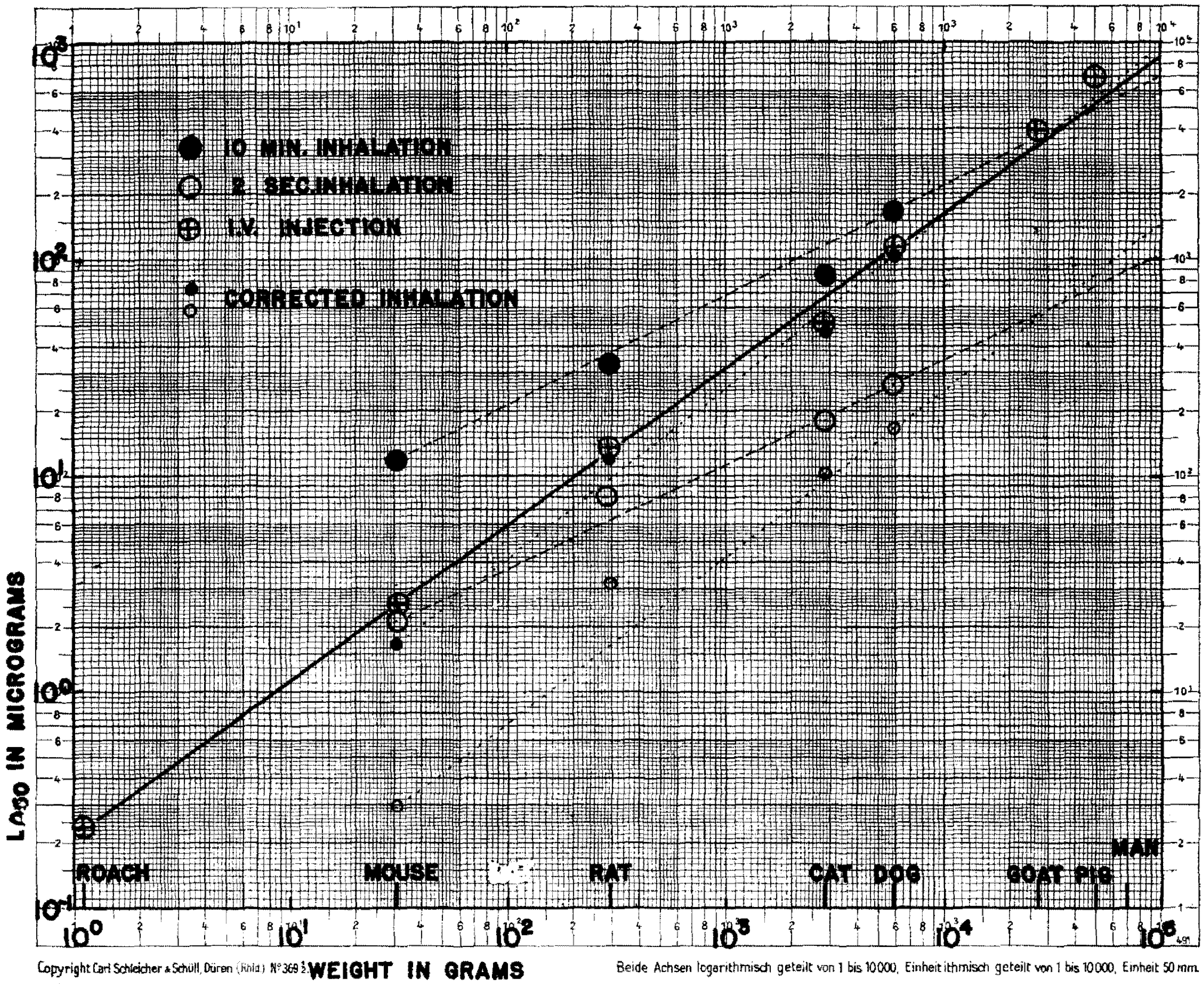
10 MIN. INHALATION
2 SEC.INHALATION
I.V. INJECTION
CORRECTED INHALATION
LD50 IN MICROGRAMS
WEIGHT IN GRAMS
ROACH
MOUSE
RAT
CAT
DOG
GOAT
PIG
MAN
Beide Achsen logarithmisch geteilt von 1 bis 10000, Einheit ithmisch geteilt von 1 bis 10000, Einheit 50 mm.

Figure 13

APPENDIX I

EXPERIMENTAL DATA

by

H. J. Trurnit

Experiments With Mice

10 Mice Per Cage

Date Temp. Rel.Hum.	Serial #	Sex	Average Weight g.	Exposure Time Sec.	Chamber Conc. mg./m.^{3}	C x t mg.min./m.^{3}	Survival Time Min. (h means hours)									
Mar. 18,	29	F	28	240	48	192	5	5	5	5	7	-	-	-	-	-
1953	30	"	30	180	48	144	7	-	-	-	-	-	-	-	-	-
	31	"	32	285	41	194	5	5	5	7	7	7	7	7	7	-
21°C.	32	"	28	210	52	182	2	5	5	5	5	5	-	-	-	-
	33	"	29	185	50	154	4	4	4	5	5	5	-	-	-	-
42%	34	"	28	165	45	124	6	8	-	-	-	-	-	-	-	-
Mar. 19,	35	"	29	60	167	167	1	1	1	1	1	1	1	1	1	-
1953	36	"	27	30	201	100	2	-	-	-	-	-	-	-	-	-
	37	"	29	45	218	162	2	2	2	2	2	2	2	-	-	-
22°C.	38	"	27	38	221	140	2	2	2	2	2	2	2	2	-	-
	39	"	26	25	224	94	2	-	-	-	-	-	-	-	-	-
50%	40	"	27	30	235	118	2	-	-	-	-	-	-	-	-	-
Mar. 24,	41	M	33	30	408	204	2	2	2	2	2	2	2	2	2	-
1953	42	"	34	20	414	138	1	1	1	1	1	1	1	-	-	-
	43	"	34	15	428	107	1	1	4	4	4	-	-	-	-	-
23°C.	44	"	34	17	426	121	2	2	2	2	2	2	-	-	-	-
	45	"	35	16	426	113	2	2	2	2	2	2	21h	-	-	-
45%	46	"	36	13	414	90	-	-	-	-	-	-	-	-	-	-
April 6,	47	"	37	7	538	63	-	-	-	-	-	-	-	-	-	-
1953	48	"	35	15	506	127	2	2	9	-	-	-	-	-	-	-
	49	"	37	20	560	187	1	1	1	1	1	1	1	1	3	-
23°C.	50	"	37	16	518	138	3	8	-	-	-	-	-	-	-	-
	51	"	36	17	470	117	2	2	2	2	2	9	10	24h	-	-
52%	52	"	33	17.5	509	148	1	1	1	1	1	1	1	2	-	-

MICE

Date Temp. Rel.Hum.	Serial #	Sex	Average Weight g.	Exposure Time Sec.	Chamber Conc. mg./m.^{3}	C x t mg.min. /m.^{3}	Survival Time Min. (h means hours)									
April 8,	53	F	31	5	654	55	-	-	-	-	-	-	-	-	-	-
1953	54	"	30	10	763	127	1	1	-	-	-	-	-	-	-	-
	55	"	29	12	788	158	1	1	1	1	10	-	-	-	-	-
26°C.	56	"	31	12.5	739	154	2	2	2	-	-	-	-	-	-	-
	57	"	27	14	773	182	2	2	2	2	2	2	3	3	3	-
50%	58	"	28	13	715	155	1	1	1	1	2	2	2	-	-	-
	59	"	32	12	715	143	1	1	-	-	-	-	-	-	-	-
													# of Deaths			
June 12,	216	M	32	2	2000	67								9		
1953	217	"	28	1	2023	34								4		
	218	"	30	1.5	2104	53	No							7		
30°C.	219	"	28	1	2028	34	Individual							6		
	220	"	32	3	1280	64	Survival							9		
	221	"	32	2	1140	38	Times Taken							5		
39%	222	"	30	1	1136	19								2		
	223	"	27	1.5	1105	28								2		
June 25,	242	F	32	8	693	92	1	2	2	-	-	-	-	-	-	-
1953	243	"	31	6	654	65	1	-	-	-	-	-	-	-	-	-
	244	"	34	10	615	103	1	-	-	-	-	-	-	-	-	-
29°C.	245	"	31	14	563	131	1	1	1	2	2	3	6^h	-	-	-
	246	"	33	12	477	96	1	1	1	10	3^h	-	-	-	-	-
	247	"	32	18	399	120	.5	.5	.5	1	1	1	2	2	-	-
63%	248	"	31	12	336	67	1	2	-	-	-	-	-	-	-	-
	249	"	37	14	266	62	2	2	-	-	-	-	-	-	-	-

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[redacted] ~~Security Information~~

Experiments With Rats

6 Rats Per Cage

Date Temp. Rel.Hum.	Serial #	Sex	Average Weight g.	Exposure Time Sec.	Chamber Conc. mg./m.^{3}	C x t mg.min./m.^{3}	Survival Time in Min. (h means hours)					
Feb. 24, 1953	1	M	292	20	158	53	-	-	-	-	-	-
	2	"	289	50	86	72	3h49	-	-	-	-	-
	3	"	322	70	65	76	-	-	-	-	-	-
23°C.	4	"	317	100	79	132	5	8	8	-	-	-
	5	"	317	85	72	102	4	7	-	-	-	-
36%	6	"	296	80	66	88	3	3	-	-	-	-
Feb. 26, 1953	7	"	324	30	180	90	4	4	4	-	-	-
	8	"	335	15	213	53	4	-	-	-	-	-
	9	"	313	20	170	57	21h	-	-	-	-	-
20°C.	10	"	367	45	199	150	3	3	3	9	9	-
	11	"	332	60	193	193	3	3	3	3	3	3
44%	12	"	318	30	191	96	6	6	6	16	-	-
Mar. 2, 1953	13	"	284	180	27	82	9	-	-	-	-	-
	14	"	226	300	39	196	11	11	11	11	-	-
	15	"	276	240	29	116	6	-	-	-	-	-
20°C.	16	"	321	420	33	234	9	9	9	13	26	-
	17	"	328	225	33	125	10	13	-	-	-	-
40%	18	"	332	270	36	160	8	8	16	-	-	-
Mar. 3, 1953	19	"	332	5	690	58	-	-	-	-	-	-
	20	"	317	15	637	160	3	4	8	-	-	-
17°C.	21	"	350	8	616	82	-	-	-	-	-	-
	22	"	358	20	708	236	4	8	8	-	-	-
30%	23	"	361	15	852	213	2	4	4	4	-	-
Mar. 17, 1953	24	"	365	5.5	1048	96	5	5	-	-	-	-
	25	"	316	12	944	189	3	3	3	3	3	13
	26	"	324	9	924	139	5	5	7	7	-	-
23°C.	27	"	343	3	879	44	-	-	-	-	-	-
40%	28	"	372	6	821	82	2	4	5	60	-	-
April 9, 1953	60	"	223	5	1001	83	5	5	5	6	-	-
	61	"	204	6	966	97	4	4	8	-	-	-
	62	"	195	8	993	132	4	4	4	4	4	4
22°C.	63	"	201	5	977	81	3	3	3	12	-	-
	64	"	171	6	976	98	3	3	5	-	-	-
50%	65	"	204	4	916	61	2	5	-	-	-	-

[redacted] ~~Security Information~~

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RATS

Date Temp. Rel.Hum.	Serial #	Sex	Average Weight g.	Exposure Time sec.	Chamber Conc. mg./m.^{3}	C x t mg.min./m.^{3}	Survival Time in Min. (h means hours)					
April 14, 1953	66	M	228	5	1035	86	3	3	4	-	-	-
	67	"	245	6	1037	104	2	2	4	4	4	4
	68	"	225	7	1019	119	3	3	3	5	5	-
	69	"	230	4	1015	68	-	-	-	-	-	-
	70	"	234	4.5	1049	79	3	3	3	3	-	-
	71	"	227	4	1067	71	5	13	5h	-	-	-
June 29, 1953	258	"	273	2	1313	44	3	4	4	5h	-	-
	259	"		3	1275	64	3	3	3	14	5h	6h
	260	"		4	1095	73	2	3	3	5	5	5
30°C.	261	"		1	1135	19	3	-	-	-	-	-
	262	"		60	92	92	8	-	-	-	-	-
	263	"		100	76	127	5	6	6	7	-	-
77%	264	"		80	74	94	4	5h	-	-	-	-
	265	"		90	71	107	5	45	5h	-	-	-

Experiments With Guinea Pigs

4 Guinea Pigs Per Cage

Date Rm. Temp. Rel. Hum.	Serial #	Sex	Average Weight g.	Exposure Time sec.	Chamber Conc. mg./m.^{3}	C x t mg.min./m.^{3}	Survival Time in Min. (h means hours)			
April 15,	72	F	413	195	70	228	8	8	9	11
1953	73	"	420	150	68	170	5	9	29	31
	74	"	390	130	69	145	8	9	-	-
22°C.	75	"	449	160	69	184	5	22h	-	-
	76	"	453	135	66	149	7	7	13	-
45%	77	"	429	120	70	140	7	17	-	-
	78	"	351	110	72	132	7	10	10	-
April 16,	79	M	447	25	220	92	5	5	7	-
1953	80	"	380	20	219	73	5	7	23	-
	81	"	475	18	233	70	15	-	-	-
23°C.	82	"	451	19	235	73	5	5	6	6
	83	"	431	18	226	68	-	-	-	-
70%	84	"	438	19	233	74	5	-	-	-
	85	"	527	17	234	66	74	-	-	-
April 20,	86	"	397	8	420	56	5	8	8	-
1953	87	"	387	12	390	78	3	3	8	33
	88	"	449	5	422	34	25	-	-	-
22°C.	89	"	514	6	431	43	-	-	-	-
	90	F	427	5	421	35	-	-	-	-
40%	91	"	390	4	450	30	-	-	-	-
	92	"	432	6	449	45	18	-	-	-
April 21,	93	M	497	6	502	50	-	-	-	-
1953	94	"	527	7	486	57	8	-	-	-
	95	"	509	6	497	50	5	43	-	-
22°C.	96	"	467	8	536	71	7	7	7	-
	97	"	310	8	485	58	6	7	-	-
40%	98	"	295	7	507	59	4	4	6	-
	99	"	313	6	457	46	-	-	-	-
April 22,	100	"	307	7	526	61	6	6	10	16
1953	101	"	323	5	517	43	-	-	-	-
	102	"	335	6	478	48	7	-	-	-
23°C.	103	"	318	6	526	53	-	-	-	-
	104	"	325	5.5	519	48	11	-	-	-
40%	105	"	325	6.5	513	56	-	-	-	-

GUINEA PIGS

Date Rm. Temp. Rel. Hum.	Serial #	Sex	Average Weight g.	Exposure Time sec.	Chamber Conc. mg./m.^{3}	C x t mg.min./m.^{3}	Survival Time in Min. (h means hours)				
April 23, 1953	106	F	275	6	564	56	7	-	-	-	
	107	"	291	7	550	64	3	-	-	-	
	108	"	290	8	572	76	3	8	13	13	
23°C.	109	"	278	7.5	543	68	4	11	-	-	
42%	110	"	265	7	556	65	9	-	-	-	
June 17, 1953	224	"	302	90	128	184	3	3	5	5	
	225	M	358	60	126	126	3	4	4	6	
	226	"	255	40	121	82	2	3	2h	-	
	227	"	397	35	125	73	12	3h	-	-	
29°C.	228	"	355	38	120	76	4	4	8	10h	
	229	"	456	3	572	29	5	-	-	-	
	230	"	444	3	502	25	8	10h	10h	-	
	231	"	464	2	337	11	-	-	-	-	
	232	"	324	3	340	17	-	-	-	-	
	233	3M 1F	378	2	176	5.8	-	-	-	-	
June 19, 1953	234	M	390	2	1220	41	5	5	-	-	
	235	"	345	1	1188	20	-	-	-	-	
	236	"	331	3	1166	58	2	4	4	10	
	237	1F 3M	295	2	900	30	7	-	-	-	
28°C.	238	F	315	2.5	756	32	10	3h	14h	-	
	239	"	308	3	590	30	10	-	-	-	
	240	"	389	1.5	510	13	-	-	-	-	
	241	2M 2F	310	4	409	27	-	-	-	-	
June 26, 1953	250		304	60	120	120	3	4	5	6	
	251		298	60	70	70	-	-	-	-	
	252		325	100	65	108	6h	-	-	-	
31°C.	253		308	130	67	145	5	6	7	-	
	254		334	100	64	105	5	5	25	-	
	255		326	85	67	94	4	13	30	-	
74%	256		334	75	65	81	12	-	-	-	
	257		258	70	64	68	6	8	-	-	

CATS

Code for Symptoms: r.d. - respiratory difficulties, s.c. - slight convulsions, h.c. - heavy convulsions, s. - salivation, m. - myosis, c. - cyanosis, d.u. - defecation and urination.

Date Rm.Temp. Rel.Hum.	Serial #	Sex	Weight kg.	Exposure Time sec.	Chamber Conc. mg./m.^{3}	C x t mg.min. /m.^{3}	Survival Time min.	Time in Min. for the Appearance of Symptoms Listed Below						
								r.d.	s.c.	h.c.	s.	m.	c.	d.u.
April 30,	111	M	3.8	20	203	68	7			1				
1953	112	"	4.1	15	212	53	104			5		15		
	113	"	3.7	10	220	37	$2^{h}36$	2	2	4		5		
23°C.	114	"	3.1	6	223	22	--	2		11		6		
	115	"	2.9	12	215	43	--		15			5		
	116	"	3.1	17	218	62	20^{h}			3				
May 5,	117	"	2.1	5	544	45	3^{h}		2	6	6	6		
1953	118	"	2.8	4	542	36	14			3		6		
	119	"	2.5	3	527	26	33		2	4		8		
27°C.	120	"	1.9	2	473	16	--	2			3	5		
	121	"	2.4	1.2	453	9	--		5			10		
	122	"	2.4	4	372	25	23^{h}			3	4			
	123	"	2.3	2	378	13	--				5			
	124	"	2.3	3	343	17	--		2		2	6		
May 6,	125	"	2.5	3	603	30	22^{h}			2	2	6		
1953	126	"	2.3	2.2	627	23	--		1			8		
	127	"	2.5	1.5	628	16	--	4				7		
27°C.	128	"	2.5	2.5	573	24	--	3				1		
	129	"	2.9	3.5	584	34	3^{h}	2		6		8		
May 7,	130	"	4.4	60	23	23	--	1				11	3	3
1953	131	"	3.6	70	26	30	--	3				6		
26°C.	132	"	3.5	90	27	41	--	5				7		
	133	"	2.0	110	29	53	--	3				6		
	134	"	2.1	150	29	73	30	4	6	7	4			
May 12,	137	"	2.4	180	21	63	18	2		4	6	5		
1953	138	"	2.5	160	23	61	--	5				5		
23°C.	139	"	2.1	180	23	69	--					7		7
	140	"	2.2	240	24	96	2^{h}	3		5	5	5		
70%	141	"	1.9	210	25	88	--	2	4	9	9	4		
May 13,	142	"	3.9	10	198	33	60	1	3	5		3		
1953	143	"	3.7	6	205	21	--	1	5			5		
26°C.	144	"	2.8	8	213	28	--	2				4		
	145	F	2.3	10	216	36	51	2	4	6	6	4		6
65%	146	M	3.1	9	222	33	--	1				5		
	147	"	3.8	20	211	70	26	1	3	6	3	6		
May 25,	171	F	2.4	60	85	85	10	2		3	3		4	
1953	172	"	2.3	40	89	60	41	4	4	6		4		
23°C.	173	"	2.0	33	93	51	$2^{h}39$	1	1	5	4	7		7
63%	174	"	1.9	20	96	32	--	2				5		
	175	"	2.0	25	101	42	--					2		1

DOGS

Date Rm. Temp. Rel. Hum.	Serial #	Sex	Weight kg.	Exposure Time sec.	Chamber Conc. mg./m.^{3}	C x t mg.min./m.^{3}	Survival Time min.	Time in Min. for the Appearance of Symptoms Listed Below: r.d.	s.c.	h.c.	s.	m.	c.	d.u.
May 14,	148	F	5.5	6	405	41	90	1	3	9	5		1	
1953	149	"	5.6	3.2	464	25	24	1	5				4	
27°C.	150	"	3.8	2	458	15	10	1						
70%	151	"	5.8	1	454	7.5	--	1						
May 18,	152	"	5.9	7	451	53	55	1	1	3	3		2	4
1953	153	"	6.0	5	440	37	15	1		12		6	2	
27°C.	154	M	6.2	4	424	28	--	12	9			4		
90%	155	"	6.5	4.8	436	35	--	2			3	7		
May 19,	156	"	5.4	2	555	18	*	1			2	4	4	
1953	157	"	5.9	3	578	29	42	1	3	15	2	6		20
24°C.	158	"	5.6	4	575	38	75	2	15	5	4	6	5	
65%	159	"	5.1	1	522	9	*							
May 20,	160	"	7.9	60	41	41	--	2				9		
1953	161	"	6.1	60	92	92	16	2		2			3	2
24°C.	162	"	5.9	35	92	54	--	2	4			16		
67%	163	"	6.1	45	96	72	100	1	4	9		9		
	164	"	6.3	40	95	63	--	1	3		7	6		
June 9,	207	F	6.3	20	167***	56	21	1	3	4	3	6	6	4
1953	208	"	5.9	15	167	42	--	1	3		5			
	209	"	5.9	17	167	47	--	1			6			
28°C.	210	M	7.6	23	167	64	13	5	2		2		6	5
	211**	"	6.3	15	167	42	--	2						
80%	212**	"	7.2	17	167	47	--	1			3			
	213**	"	8.1	23	167	64	--	5			3			
	214**	"	6.8	27	167	75	--	1	2		5	5		
	215**	F	5.9	35	167	97	--	1	3		2	5	5	

* These two dogs died 6 and 15 days later.

** These dogs were reexposed survivors with the original serial numbers of 211-164, 212-162, 213-160, 214-154 and 215-151.

*** In this series only two analytical samples were taken from the chamber, one at the beginning, one near the end of series. These two samples gave values of 169 and 165 mg./m.^{3}.

MONKEYS

Date Rm. Temp. Rel. Hum.	Serial #	Sex	Weight kg.	Exposure Time sec.	Chamber Conc. mg./m.^{3}	C x t mg.min./m.^{3}	Survival Time min.	Time in Min. for the Appearance of Symptoms Listed Below r.d.	s.c.	h.c.	s.	m.	c.	d.u.
May 21,	165	M	3.1	2	337	11	1		1					1
1953	166	"	2.9	2	147	4.9	9		1			4		1
	167	F	2.9	2	155	5.2	-	5	5			15		
26°C.	168	"	3.3	5	153	13	8	1		2				
	169	M	3.5	3	131	6.6	4	1		1				1
60%	170	"	3.2	2	129	4.3	8	2	4	2		3		
May 26,	176	M	-	10	233	40	3	1	2					
1953	177	"	-	6	254	25	8	1	3	2		4	3	
	178	"	2.7	10	121	20	-	4	12			4		
23°C.	179	F	-	20	112	37	4	2						
	180	"	-	12	115	23	8	1		2	6	3	6	
72%	181	M	-	10	119	20	6	1		2		1	2	1
May 27,	182	F	2.7	60	41	41	14		9	5	2	7		
1953	183	M	2.9	40	44	29	5	1	7	1				
	184	F	3.3	40	35	24	-	2	6	7		3		
24°C.	185	M	3.1	35	34	20	-	1				2		
54%	186	"	-	50	33	28	4	1	2	1				
	187	F	2.3	40	33	22	-	1	4			3		
May 28,	188	"	2.5	4	230	15	-	1	3			3		
1953	189	"	3.2	6	213	21	-	3		3		3		
	190	M	2.7	8	210	28	-	1				2		
22°C.	191	"	2.3	4	305	20	-	1				2		
	192	"	3.6	8	298	40	3	1		2	2		2	
45%	193	F	2.7	6	300	30	4	1		2		2		
	194	"	3.2	5	303	25	5	1		2		3		
May 29,	195	"	3.6	3	455	23	11	1	7	4		2		
1953	196	"	3.2	6	468	47	3			1				
	197	M	3.5	2.5	477	20	4	1		2		2		2
22°C.	198	"	3.7	4	370	25	2	1		1				
	199	"	4.2	1	356	5.9	7	1	2	3		3		
50%	200	"	3.8	2	254	8.5	9	1	3	3		2	4	
	201	"	3.4	1	233	3.9	-	1				4		
June 5,	202	F	3.2	500	1.8	15	-							*
1953	203	"	4.4	270	8.7	39	7				5		6	*
28°C.	204	M	3.8	210	8.9	31	14	8				4	7	
	205	"	3.6	170	9.0	25	-	5	3			3		
62%	206	F	4.2	183	9.9	30	8	3		1			3	

* These monkeys developed myosis, heavy convulsions and respiratory difficulties while in the chamber.

APPENDIX II

POST MORTEM REPORT

by

[redacted]

Mice

Post-mortem No. 84-53

Date Completed: 10 April 1953

History or experimental details:

Preamble:

This project was undertaken by Pathology as routine service work in connection with so-called "crash operation". Neither the object of this work, nor the techniques, were our concern. In essence, Pathology was asked to undertake routine post mortem examination of all animals used in this project to determine principally whether any intercurrent disease was present in groups of animals, of sufficient extent and severity, which would have interfered with the specific toxicity of the experimental treatment.

Results - Mice.

All mice, dead on arrival (D.O.A.) or alive (and then killed) presented to us, therefore, were subjected to the usual anatomical examination. Observations were restricted to gross appearances of organs and tissues. Unless where otherwise stated, all the following mice must be regarded as normal. In order that relationship with experimental groups can be established, the mice have been identified in these reports by the appropriate experimental number given to us, and the date of the autopsies.

March 18, 1953 ...	Serial	number	29 ...	five mice.	No	abnormalities.	D.O.A.
	"	"	30 ...	one mouse.	"	"	"
	"	"	31 ...	ten mice.	"	"	"
	"	"	32 ...	six mice.	"	"	"
	"	"	33 ...	six mice.	"	"	"
	"	"	34 ...	two mice.	"	"	"
March 19, 1953 ...	"	"	29 ...	five mice.	"	"	Living
	"	"	30 ...	ten mice.	"	"	"
	"	"	32 ...	four mice.	"	"	"
	"	"	33 ...	four mice.	"	"	"
	"	"	34 ...	eight mice.	"	"	"
	Controls		...	ten mice.	"	"	"
March 19, 1953 ...	Serial	number	35 ...	nine mice.	No	abnormalities	D.O.A.
	"	"	36 ...	one mouse.	"	"	"
	"	"	37 ...	seven mice.	"	"	"
	"	"	38 ...	eight mice.	"	"	"
	"	"	39 ...	one mouse.	"	"	"
	"	"	40 ...	one mouse.	"	"	"

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March 20, 1953 ...	Serial	number	35 ...	one mouse.	No	abnormalities.	Living
	"	"	36 ...	nine mice.	"	"	"
	"	"	37 ...	three mice.	"	"	"
	"	"	38 ...	two mice.	"	"	"
	"	"	39 ...	nine mice.	"	"	"
	"	"	40 ...	nine mice.	"	"	"
March 24, 1953 ...	"	"	41 ...	nine mice.	"	"	D.O.A.
	"	"	42 ...	seven mice.	"	"	"
	"	"	43 ...	five mice.	"	"	"
	"	"	44 ...	six mice.	"	"	"
	"	"	45 ...	six mice.	"	"	"
March 25, 1953 ...	"	"	41 ...	one mouse.	"	"	Living
	"	"	42 ...	three mice.	"	"	"
	"	"	43 ...	four mice.	"	"	"
	"	"	44 ...	four mice.	"	"	"
	"	"	45 ...	four mice.	"	"	3 Living 1 D.O.A.
	"	"	46 ...	ten mice.	"	"	Living
April 6, 1953 ...	"	"	48 ...	three mice.	"	"	D.O.A.
	"	"	49 ...	nine mice.	"	"	"
	"	"	50 ...	two mice.	"	"	"
	"	"	51 ...	seven mice.	"	"	"
	"	"	52 ...	eight mice.	"	"	"
April 7, 1953 ...	"	"	47 ...	ten mice.	"	"	Living
	"	"	48 ...	seven mice.	"	"	"
	"	"	49 ...	one mouse.	"	"	"
	"	"	50 ...	eight mice.	"	"	"
	"	"	51 ...	two mice.	"	"	"
	"	"	52 ...	two mice.	"	"	"
April 8, 1953 ...	"	"	54 ...	two mice.	"	"	D.O.A.
	"	"	55 ...	five mice.	"	"	"
	"	"	56 ...	three mice.	"	"	"
	"	"	57 ...	nine mice.	"	"	"
	"	"	58 ...	seven mice.	"	"	"
	"	"	59 ...	two mice.	"	"	"
April 9, 1953 ...	"	"	53 ...	nine mice.	"	"	Living
	"	"	54 ...	eight mice.	"	"	"
	"	"	55 ...	five mice.	"	"	"
	"	"	56 ...	seven mice.	"	"	"
	"	"	57 ...	one mouse.	"	"	"
	"	"	58 ...	three mice.	"	"	"
	"	"	59 ...	eight mice.	"	"	"

<u>Comments</u>:

There was no evidence in any of this batch of mice of specific intercurrent disease, which would have had any significant influence on the experimental gassing procedure.

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Rats
Post-mortem No. 84-53
Date Completed: 16 June 1953

History or experimental details:

Results - Rats:

These animals were subjected to the usual routine examination.

Date, P.M.	Serial number	Findings
Feb. 24, 1953		
D.O.A.	4	1 Parasitic liver.
		2 No lesions.
	5	1 Pneumonia
		1 Parasitic liver.
	6	2 No lesions.
Feb. 25, 1953	Controls	4 No lesions.
Living		4 Parasitic liver.
	1	2 No lesions.
		3 Parasitic liver.
		1 Pneumonia.
	2	2 No lesions.
		4 Parasitic liver.
	3	3 No lesions.
		3 Parasitic liver.
	4	1 Parasitic liver.
		2 No lesions.
	5	1 Parasitic liver.
		3 No lesions.
	6	1 Parasitic liver.
		3 No lesions.
Feb. 26, 1953		
D.O.A.	7	2 No lesions.
		1 Parasitic liver.
	8	1 Parasitic liver.
	10	5 No lesions.
	11	4 No lesions.
		2 Parasitic liver.
	12	2 No lesions.
		2 Parasitic liver.
		1 Pneumonia.
Feb. 27, 1953		
Living	7	2 No lesions.
		1 Parasitic liver.
	8	6 No lesions.
	9	4 No lesions.
		2 Parasitic liver.

Feb. 27, 1953		
Living	10	1 No lesions.
	12	2 No lesions.
March 2, 1953		
D.O.A.	13	1 No lesions.
	14	2 No lesions.
		1 Parasitic liver.
		1 Pneumonia.
	15	1 Parasitic liver.
	16	2 No lesions.
		3 Parasitic liver.
	17	1 Parasitic liver.
	18	2 Parasitic liver.
		1 No lesions.
March 3, 1953		
Living	13	5 No lesions.
	14	1 No lesions.
		1 Parasitic liver.
	15	4 No lesions.
		1 Parasitic liver.
	16	1 No lesions.
	17	4 No lesions.
	18	2 No lesions.
		1 Parasitic liver.
	20	3 No lesions.
	22	3 No lesions.
	23	2 No lesions.
		2 Parasitic liver.
March 4, 1953		
Living	19	3 Parasitic liver.
		3 No lesions.
	20	2 No lesions.
		1 Parasitic liver.
	21	4 No lesions.
		2 Parasitic liver.
	22	1 Pneumonia.
		2 Parasitic liver.
	23	2 Pneumonia.
March 17, 1953		
D.O.A.	24	2 No lesions.
	25	4 No lesions.
		2 Parasitic liver.
	26	4 Parasitic liver.
		1 Pneumonia.
		1 No lesions.
	28	4 No lesions.
March 18, 1953		
Living	24	4 Parasitic liver.
	26	1 Parasitic liver.
		1 No lesions.

March 18, 1953 Living	27	2 Parasitic liver.
		4 No lesions.
	28	1 Parasitic liver.
		1 No lesions.
April 9, 1953 D.O.A.	60	4 No lesions.
	61	3 No lesions.
	62	4 No lesions.
		1 Parasitic liver.
		1 Pneumonia.
	63	3 No lesions.
		1 Pneumonia.
	64	3 No lesions.
	65	2 No lesions.
April 10, 1953 Living	60	1 No lesions.
		1 Parasitic liver.
	61	2 No lesions.
		1 Parasitic liver.
	63	2 No lesions.
	64	3 Parasitic liver.
	65	2 Parasitic liver.
		2 No lesions.
April 14, 1953 D.O.A.	66	2 No lesions.
		1 Parasitic liver.
	67	5 No lesions.
		1 Parasitic liver.
	68	4 No lesions.
		1 Parasitic liver.
	70	3 No lesions.
		1 Pneumonia.
	71	2 Parasitic liver.
April 15, 1953 Living	66	1 Pneumonia.
		2 No lesions.
	68	1 Pneumonia.
	69	5 No lesions.
		1 Parasitic liver.
	70	2 No lesions.
	71	3 No lesions.
		1 Pneumonia.

<u>Comments</u>:

There was tremendous variation in the findings of individual groups of animals, so that it is impossible to make any sweeping generalization about their condition and the effect of all the incidental lesions on acute toxicity. Single parasitic cysts of the liver are extremely common in our bought-in rats, and it is very doubtful if this lesion would have any significant influence on acute toxicity of the compound used. The problem of the focal pneumonitis is another matter; pneumonia in rats is rather like the analagous condition in guinea pigs. It may lie dormant for a long time, permit of normal body growth, and cause no active progressive pneumonia involving whole lobes or even all the lungs. In this case possibly judgment may have to be made on your own figures of dose and survival of different groups.

Guinea Pigs
Post-mortem No. 84-53
Date Completed: 16 June 1953

History or experimental details:

The guinea pigs were subjected to the routine examination given all the different species in this whole experiment. Our examination was restricted to a definition of either normality, or gross lesions affecting the important viscera of the thorax and abdomen.

Results - Guinea pigs.

Date P.M.	Serial number	Findings
April 15, 1953		
D.O.A.	72	4 with pneumonia.
	73	2 with pneumonia.
		1 pregnant.
		2 no lesions.
	74	2 with pneumonia.
	75	1 with pneumonia.
	76	2 with pneumonia.
		1 no lesions.
	77	2 with pneumonia.
		1 pregnant.
	78	3 with pneumonia.
		1 with peritonitis.
April 16, 1953		
Living	74	1 with pneumonia.
		1 no lesions.
	75	2 with pneumonia.
		1 normal. No lesions.
		1 pregnant.
	76	1 with pneumonia.
	77	2 with pneumonia.
	78	1 with pneumonia.
April 16, 1953		
D.O.A.	79	2 with pneumonia.
		1 no lesions.
	80	3 with pneumonia.
	81	1 with pneumonia.
	82	4 with pneumonia.
	84	1 with pneumonia.
	85	1 with pneumonia.
April 17, 1953		
Living	79	1 with parasitic liver-one cyst.
	80	1 with pneumonia and parasitic liver - one cyst.

April 17, 1953		
Living	81	3 with pneumonia.
	83	3 with pneumonia.
		1 no lesions.
	84	3 with pneumonia.
	85	2 with pneumonia.
		1 no lesions.
April 20, 1953		
D.O.A.	86	3 with pneumonia.
	87	4 with pneumonia.
		1 with pericarditis caused by purulent pneumonia.
	88	1 with pneumonia.
	92	1 with pneumonia.
April 21, 1953		
Living	86	1 with pneumonia.
	88	3 with pneumonia.
	89	3 with pneumonia.
		1 no lesions.
		1 pregnant.
	90	2 with pneumonia.
		2 no lesions.
	91	3 with pneumonia, 1 case involving pleuritis.
		1 no lesions.
	92	2 with pneumonia.
		1 no lesions.
April 21, 1953		
D.O.A.	94	1 no lesions.
	95	2 with pneumonia - caseous whole lobe.
	96	2 with pneumonia.
		1 no lesions.
	97	1 with pneumonia.
		1 no lesions.
	98	2 with pneumonia.
		2 no lesions.
April 22, 1953		
D.O.A.	100	2 with pneumonia.
		2 no lesions.
	102	1 no lesions.
	104	1 no lesions.
April 22, 1953		
Living	93	4 with pneumonia.
	94	3 with pneumonia.
	95	2 with pneumonia.
		1 no lesions.
	96	1 no lesions.
	97	2 no lesions.

April 22, 1953		
Living	98	1 no lesions.
	99	3 with pneumonia.
		1 with parasitic liver-one cyst.
April 23, 1953		
Living	101	2 with pneumonia.
		2 no lesions.
	102	1 with pneumonia.
		2 no lesions.
	103	2 with pneumonia.
		2 no lesions.
	104	3 with pneumonia.
	105	4 with pneumonia.
April 23, 1953		
D.O.A.	106	1 no lesions.
	107	1 no lesions.
	108	1 with pneumonia.
		3 no lesions.
	109	2 no lesions.
	110	1 with pneumonia.
April 24, 1953		
Living	106	1 with pneumonia.
		2 no lesions.
	107	2 with pneumonia.
		1 no lesions.
	109	2 with pneumonia.
	110	1 with pneumonia.
		2 no lesions.

Comments:

A survey of the above tables will indicate that over 70 per cent of the experimental animals suffered from various grades of some form of pulmonary disease. This condition is the common endemic lung disorder of guinea pigs seen so frequently in animals in the Med Labs bought in from commercial sources. It has a variety of bacteria associated as causal organisms. It may remain clinically silent for a long period; it is impossible to determine whether any given pig suffers from this simply by "clinical" observation of the living animal. In these latter type of animals the lesions may be composed of small areas of consolidation affecting a tip or border of one lobe, or it may affect a whole lobe. In both these grades the animals may live for a long time with no evidence of ill health at all, unless subjected to some severe chronic experiment. In the very severe type the process may become caseous, purulent, and may affect pleura and pericardium by contiguous spread of the infection. Most of the pigs in the crash operation belong to the former category, i.e. they showed small areas of pneumonia, or a whole apical or intermediate lobe was affected. We don't know whether this would affect the toxicity of the compound used. This may be determined by your examination of the results of different experiments, and a comparison of survival periods of animals showing no lesions.

Cats
Post-mortem No. 84-53
Date Completed: 16 June 1953

History or experimental details:

Routine autopsies were performed on all animals submitted dead or alive. All living animals were killed with ether.

Date P.M.	Serial number	Weight of animals
April 17, 1953		
D.O.A.	134	2120 gms.
April 30, 1953		
D.O.A.	111	----
	112	----
	113	----
May 1, 1953		
Living	114	----
	115	----
	116	----
May 5, 1953		
Living	121	2520 gms.
	120	1410 "
	123	2325 "
	124	2250 "
	129	2900 "
	122	2440 "
May 5, 1953		
D.O.A.	117	2120 "
	118	2750 "
	1199	2500 "
May 7, 1953		
Living	125	----
	126	----
	127	----
	128	----
May 8, 1953		
Living	130	4400 "
	133	2020 "
	131	3670 "
	132	3520 "
May 12, 1953		
D.O.A.	137	2400 "
	140	2180 "
May 13, 1953		
Living	141	1950 gms.
	139	2070 "
	138	2530 "

May 13, 1953		
D.O.A.	147	3840 gms.
	145	2340 "
	142	3910 "
May 14, 1953		
Living	144	2830 "
	146	3160 "
May 18, 1953		
Living	143	3700 "
May 25, 1953		
D.O.A.	171	2390 "
	172	2300 "
	173	2040 "
May 26, 1953		
D.O.A.	174	1940 "
	175	2010 "

Comments:

There were no specific lesions in any of the viscera of the thoracic or abdominal cavity which could be regarded as representative of any common intercurrent disease, and which would have influenced the results of any acute toxicity procedure.

Dogs
Post-mortem No. 84-53
Date Completed: 16 June 1953

History or experimental details:

All of the animals listed below were brought in dead for routine gross pathological examination. No macroscopic lesions were noted on the external surface of the body, nor in any of the thoracic and abdominal viscera.

Date P.M.	Serial number	Weight of animal
May 14, 1953	148	5560 gms.
	149	5640 "
	150	3860 "
May 18, 1953	152	6000 "
	153	5940 "
May 19, 1953	156	6050 "
	157	5900 "
May 20, 1953	161	6150 "
	163	6100 "
June 4, 1953	164	5890 "
June 9, 1953	207	6290 "
	210	7600 "

Comments:

All the dogs can be regarded having been in good body condition, and without any evidence of important diseases affecting the throacic or abdominal viscera. There was certainly no evidence of the presence of lesions betokening the common bacterial or virus diseases of dogs.

Monkeys
Post-mortem No. 84-53
Date Completed: 16 June 1953

History or experimental details:

Results, Monkeys:

All monkeys were subjected to usual routine examination. The Pathology Branch is interested in pulmonary pathology of primates; particular attention was, therefore, directed to the lungs, and in many animals portions were taken for histological examination. This was not done to determine effects of the experimental treatment, and consequently no observations are made on the histological appearances of the lungs.

Date P.M.	Serial number	Weight of animals
May 21, 1953	169	3540 gms.
	170	3150 "
	168	3340 "
	X	3090 "
	XX	2920 "
May 22, 1953	167	2940 "
May 26, 1953	180	—
	181	—
	177	—
	176	—
	179	—
May 27, 1953	178	2680 gms.
	182	2740 "
	183	2940 "
	186	2750 "
May 28, 1953	192	3620 "
	193	2650 "
	194	3200 "
May 29, 1953	198	3700 "
	199	4200 "
	200	3820 "
	195	3570 "
	196	3150 "
May 29, 1953	184	3310 "
	185	3110 "
	187	2280 "
June 5, 1953	204	3760 "
	206	4190 "
	203	4400 "

<u>Comments</u>:

In general, this batch of monkeys can be regarded as good specimens of healthy animals. There was no evidence of any specific intercurrent disease affecting thoracic or abdominal viscera which would have had any significant influence on the experimental gassing treatment. We exclude from this statement the problem of Pneumonyssus (mite) infestation of lungs. No monkey in the above group failed to show lesions caused by this mite. In some only 2-3 minute lesions (about 2 mm. in diameter) were present, in some perhaps up to a dozen were counted. These lesions are a universal finding in Rhesus monkeys imported into this country. The lesions are focal and usually remain restricted to the site of mite lodgement deep in lung tissue. It is rare to find this process extending into a lobar, or more generalized, pulmonary disease; indeed we have yet to find a single case of the latter. In view of this we think it is doubtful if these lesions would have any effect whatsoever on the toxicity level of given compounds which are highly lethal in a few hours. Curiously, whatever toxicity the chemical compound has on mammalian tissue, we had no difficulty in picking out live mites from the lung lesions.

APPENDIX III

by

[redacted] (b) (6)

A. THE Lct_{50}* FROM EXPOSURES OF SINGLE ANIMALS

Usually to obtain toxicity data of a compound a multiple number of animals are exposed simultaneously; however, the use of large animals sometimes permits only a single animal per exposure. After the responses and doses (ct) in a sufficient number of single animals have been observed, a reasonable estimate of the median lethal dose (Lct_{50}) can, perhaps, be made. However, it is very difficult to make estimates of the slope of the dose mortality curve from experimentations of this type, Finney (1).

Muir et al. (2) estimated the LCt_{50} (t = ½ min.) of GB vapor and two therapeutic treatments to monkeys from exposures of single animals by the method outlined by Finney, however, confidence limits of the estimate could not be made since the slope of the dose-mortality curve was unstable.

In some recent toxicity studies at Cml C Medical Laboratories in which single exposures were made on cats, dogs, and monkeys, the Discriminant Function (3) was used to estimate the median lethal dosage (Lct_{50}) and its confidence limits over the range of c's and t's used.

<u>Application of the Discriminant Function to Single Exposures of Animals.</u>

The chamber concentration (c) in mg./m.^{3} and exposure time (t) in minutes were both varied and an animal at the particular c and t was observed to live or die. The following equation says that the constant of proportionality equals some function of the concentration times some function of the exposure time. When the constants** are known, any c and t satisfying the relation will produce death by inhalation in half the animals exposed.

$$K = c^{\lambda_1} t^{\lambda_2} \tag{1}$$

This can be written in the logarithmatic form as follows:

$$K' = \lambda_1 c' + \lambda_2 t' \tag{1a}$$

c' and t' are logarithms of the c and t
K' is a constant in logarithms.

* Lct_{50}, instead of LCt_{50}, is used here to indicate both concentration and exposure time were variables.

** When $\lambda_1 = \lambda_2$ then any increase or decrease in one of the variables has the effect of the equivalent decrease or increase in the other. Whereas, when $\lambda_1 \neq \lambda_2$ then one of the variables may be more important than the other in producing the desired effect. For example, if λ_1 is twice as large as λ_2, i.e., $\lambda_1 = 2\lambda_2$, then a doubling of the concentration (associated with λ_1) would be just as effective as a fourfold increase in the exposure time (associated with λ_2).

Estimates of λ_1 and λ_2 are obtained by solving the normal equations set up from the observed data:

$$\left.\begin{aligned} Sc'^2 \lambda_1 + Sc't' \lambda_2 &= d_1 \\ Sc't' \lambda_1 + St'^2 \lambda_2 &= d_2 \end{aligned}\right\} \qquad (2)$$

where d_1 and d_2 are the average differences in the Log concentrations and Log exposure times of the two types of responses, and Sc'^2, St'^2 and $Sc't'$ are the sum of squares and products of the observed c's and t's.

K' is obtained from the following relation:

$$K' = \frac{\alpha_1 + \alpha_2}{2} \qquad (3)$$

where α_1 is the average k_1 from the individual c's and t's of the animals that died,
and α_2 is the average k_2 from the individual c's and t's of the animals that lived.

Significance of the Discriminant Function:

The next question to be tested is: "does the observed data suggest that a significant Discriminant Function (D) exists?" where:

$$D = \lambda_1 d_1 + \lambda_2 d_2 \qquad (4)$$

The test of significance is by the following analysis of variance:

Source	Degrees of Freedom	M.S.
Total	n - 1	
Between Dead and Alive	2	$\frac{1}{2}\left(\frac{N_1 N_2}{N_1+N_2}\right)^{*} D^2$
Within Groups	n - 3	D/n-3

where the F ratio of between dead and alive to within groups mean squares must be significant if a discriminant function exists and then

$$s^2 = \frac{D}{n-3} \qquad (5)$$

Variance of K'

From the method of discriminant function, it can be shown that:

$$V(\alpha_1 - \alpha_2) \text{ or } V(D) = s^2$$

but

$$V(\alpha_1 - \alpha_2) = V(\alpha_1 + \alpha_2)$$

and

$$V\left(\frac{\alpha_1 + \alpha_2}{2}\right) = \frac{s^2}{4}$$

* N_1 and N_2 are the observed number of animals dead and alive.

therefore

$$V(K') = \frac{s^2}{4} \qquad (6)$$

where s^2 is the mean square within group error from the Analysis of Variance of the Discriminant Function.

<u>Use of the Discriminant Function on the Cat Data</u>:

The actual mechanics of establishing the characteristic relation of the Lct_{50} to exposure time ($Lct_{50} = Kt^n$) by the use of the discriminant function is given below. When the form that corresponds to equation (1):

$$54 = c.t^{0.73}$$

or

$$c = \frac{54}{t^{0.73}}$$

is known.

Then Lct_{50} as a function of exposure time is given by multiplying both sides of the above equation by t thus corresponding to $Lct_{50} = Kt^n$:

$$ct = 54t^{0.27}$$

The observed data of the cat exposures is given in Appendix I, the logarithms of which are shown in Table 1 along with the statistics necessary to fit the constants to equation (1) in terms of Logarithms.

In solving for the λ's, the normal equations shown in equation (2) are:

$$10.07\ \lambda_1 - 12.84\ \lambda_2 = 0.1635$$
$$-12.84\ \lambda_1 + 17.84\ \lambda_2 = 0.0363$$

therefore

$$\lambda_1 = 0.2292$$
$$\lambda_2 = 0.1670$$

The Discriminant Function of equation (4) is therefore:

$$D = (.2292)\ (.1635) + (.1670)\ (.0363) = .04354$$

Its significance is tested as follows:

Source of Variation	Degrees of Freedom	S.S.	Mean Square
Between Dead and Alive	2	.01625(*)	.008125**
Within Groups	35	.04354	.001361

(*) $\left(\frac{N_1 N_2}{N_1 + N_2}\right) D^2$

** Giving an F value of 5.96 which is significant at less than the P = .01 level and s^2 is estimated to be .001360 (in terms of logs).

In solving for K' by use of equation (3)

$$K' = \frac{.7529 + .7094}{2} = .7312$$

where $\alpha_1 = .2292(2.2940) + .1670(1.3593) = .7529$

$\alpha_2 = .2292(2.1305) + .1670(1.3230) = .7094$

By equation (6):

The $V(K') = \frac{s^2}{4} = \frac{.001360}{4} = .00034$

The S.E. $K' = \sqrt{.00034} = .0184$

and the 19/20 confidence limits of K' =

$(2.04)(.0184) = .0375$

where 2.04 is the value of student's t with 32 degrees of freedom.

In terms of logarithms, the relation of c' and t' to yield a 50% mortality in these experiments in cats is:

$$.7312(.6937 \text{ to } .7687) = .2292c' + .1670t'$$

Dividing through by 0.2292 yields

$$3.1902(3.0266 \text{ to } 3.3538) = c' + 0.7286\,t'$$

Reconverting to Anti-Logarithms:

$$1550(1063 \text{ to } 2260) = c\,t^{0.73}$$

Since t was carried as (100t) to eliminate negative logarithms, the final equation is:

$$54(37 \text{ to } 80) = c\,t^{0.73}$$

Any combination of concentrations (mg./m.^{3}) and exposure times (min.) that satisfy this equation will yield a mortality in half the cats exposed.

In addition to the data on cats shown above, the data on dogs and monkeys given in Appendix I and three sets of single exposure data on monkeys observed by Muir, et al. (2), were all analyzed by the method of discriminant functions, and the results are given in Table II. Only the data for cats and the monkeys of Muir which received a .0285 injection of atropine met the requirements for being significantly discriminant.

A comparison of the LCt_{50}'s obtained by Muir et al. is made in the table below with the Lct_{50} estimated by the discriminant function method; the agreement between the two is excellent.

	Computed by Discriminant Functions Lct_{50} t = .5 min.	Reported by Muir LCt_{50} t = .5 min.
Monkeys (no treatment)	64	65
Monkeys (.0285 mg./kg. atropine)	133	124
Monkeys (.0427 mg./kg. atropine)	133	134

Table III indicates the expected Lct_{50}'s for cats at various exposure times and a comparison with LCt_{50}'s in cats from other sources.

The use of the Discriminant Function to estimate the Lct_{50} characteristic curve (in relation to exposure time) of inhalation toxicities from single animal exposures when both the chamber concentration and exposure time are varied is only an approximate method. Its use from a theoretical standpoint to obtain valid estimates of error for the curve has not been investigated completely, however, it does substantiate estimates of the constants that have been satisfactorily obtained by other accepted methods.

B. EVALUATION OF THE CONSTANTS K and n FOR MULTIPLE EXPOSURES

From the observed data of guinea pigs, rats and mice, shown in Appendix I, the LT_{50} was estimated by the probit analysis method, Finney (1). The LT_{50} having been computed was paired with its average concentration. This was done for each species separately. From these paired values the concentration was used as the dependent variable and LT_{50} as the independent variable to obtain the estimates of the constant of proportionality (K) and exponents of the variables c* and t as shown below:

$$c = Kt^{n'} \qquad (1)$$

where c was the chamber concentration and t the LT_{50}**.

Multiplying both sides of equation (1) by t gives an estimate of the Median Lethal Dose:

$$Lct_{50} = Kt^{n}{}^{***}$$

The method of least squares was used in evaluating the constants K and n'. Not all the data shown in Appendix I for rats, mice, and guinea pigs could be used, since in some dose-mortality curves valid estimates of the LT_{50} could not be obtained and also some of the dose-mortality curves were eliminated because of known abnormalities in the experimentation. The data used for evaluating K and n' is shown in Table IV.

Tests of significance showed that in the guinea pig, mouse and rat data, n was significantly less than unity; this indicates that the Median Lethal Dose increases relative to an increase in the exposure time.

* c has unity for its exponent.
** The LT_{50} was taken as the exposure time.
*** n was obtained by adding n' to unity, i.e., n = 1 + n'.

REFERENCES

1. Finney, D. J. 1947. Probit Analysis, Cambridge at the University Press.

2. Muir, Caloway. 1952. Studies in the Therapy of G Poisoning, PTP 300.

3. Fisher, R. A. 1941. Statistical Methods for Research Workers, p. 279, 8th Edition, G. E. Stechert & Co., New York.

TABLE I

Logarithms of cs and ts from the Cat Exposures to GB as Given in Appendix I

	Cats Dead		Cats Alive	
	c' mg./m.^{3} Log c	t' Min. Log (100t)	c' mg./m.^{3} Log c	t' Min. Log (100t)
	2.31	1.52	2.35	1.00
	2.33	1.46	2.33	1.30
	2.35	1.23	2.34	1.45
	2.74	.90	2.67	.48
	2.73	.85	2.66	.30
	2.72	.70	2.58	.48
	2.57	.85	2.54	.70
	2.78	.70	2.80	.60
	2.77	.78	2.80	.30
	1.46	2.40	2.76	.60
	1.32	2.48	1.36	2.00
	1.38	2.60	1.42	2.07
	2.30	1.23	1.43	2.18
	2.33	1.23	1.46	2.26
	2.32	1.52	1.36	2.43
			1.36	2.48
			1.40	2.54
			2.31	1.00
			2.33	1.11
			2.35	1.18
Av.	2.2940	1.3593	2.1305	1.3230

$N_1 = 15$ $N_2 = 20$

$d_1 = 2.2940 - 2.1305 = 0.1635$

$d_2 = 1.3593 - 1.3230 = 0.0363$

$Sc'^2 = 10.0700$

$Sc'^2 = 17.8408$

$Sc't' = 12.8413$

TABLE II

Estimates of the Lct_{50} from Exposures of Single Animals

Animals	Characteristic Curve for Lct_{50} as Function of Exposure Time	Lct_{50} mg. min./m.^{3} t = .5 min.	Discriminant Function
Cats (35)	$54^b\ t^{0.27}$	42 ± 10	Significant P < .01
Dogs (21)	$79^c\ t^{0.42}$	59	Not Significant
Monkeys (37)	$53^d\ t^{0.66}$	34[a]	Not Significant
Monkeys, no treatment (10)	NA	64[a]	Not Significant
Monkeys, .0285 mg./kg. atropine (21)	NA	133 ± 14	Significant P < .01
Monkeys, .0427 mg./kg. atropine (13)	NA	133[a]	Not Significant

( ) indicate the number of animals used.
NA indicates characteristic curve is not available since only one level of exposure time was used.
a No confidence limits available.
b The S.E. of this statistic is about ± 11.
c The S.E. of this statistic is not available.
d The S.E. of this statistic is not available.

TABLE III

Comparison of Expected Lct_{50}'s (from $54t^{0.27}$) with Those Observed in Other Experiments

Time of Exposure in min.	$Lct_{50} = 54t^{0.27}$ mg. min./m.^{3}	LCt_{50}** (other labs) mg. min./m.^{3}
10	100 ± 25*	ca 100, 79
5	83 ± 16	
4	79 ± 15	70
1	54 ± 11	49
0.16	33 ± 8	42
.03	21	
.01	16	

All values below and above .03 and 4.0 minutes are extrapolated.

* ± 1 S.E.

** Cited by F. P. McGrath et al. 1952. Acute inhalation toxicity of GA and GB vapors to cats exposed for ten minutes. MLRR 136.

TABLE IV

Data from Appendix I used for Evaluating K and n in $Lct_{50} = Kt^{n}$

Ser. #	Av. Concn. mg./m.^{3}	LT_{50} min.
	Mice ct = 169 $t^{0.34}$	
29-34	47	3.49
35-40	211	.63
41-46	421	.22
47-52	522	.32
242-245	631	.21
53-59	735	.21
220-223	1165	.03
216-219	2039	.02
246-249	omitted for experimental reasons	
	Guinea Pigs Lct_{50} = 100 $t^{0.27}$	
72-78	69	1.97
224-228	124	.56
79-85	229	.33
86-92	426	.12
93-99	497	.11
106-110	560	.13
100-105	omitted for heterogenous data	
229-233	omitted for heterogenous data	
234-241	omitted for experimental reasons	
250-257	omitted for experimental reasons	
	Rats Lct_{50} = 112 $t^{0.20}$	
13-18	33	3.88
1-6	88	1.43
7-12	191	.45
24-28	923	.10
60-65	972	.08
66-71	1037	.08
19-23	omitted for experimental reasons	
258-265	omitted because mortality curve was hetergenous	

APPENDIX IV

Remarks on Wall Adsorption of GB

by

H. J. Trurnit

It was mentioned under "Procedure" that the time during which the animals stayed in the exposure compartment before and after the actual exposure was cut down to a minimum.

Originally, one of the concepts which lay behind the special design of the chamber was the possibility of permitting the animal to adapt to the new environment before the actual experiment. It was thought that excitement affecting the breathing pattern and pulse rate would have subsided during this interval before the actual exposure. However, preliminary tests with dogs (see special report by (b) (6) [redacted] [redacted]) and our own observations of the behavior of the other species showed that the pattern of behavior including breathing did not change much after entering the exposure compartment. In fact, it was later thought to be an advantage if the animals moved around as much as possible during the exposure in order to "sample" an average chamber concentration. There was always a slight possibility that the concentration near the walls and in the corners of the compartment deviated from that in the center.

During the first few weeks of operation, it was observed that after starting the chamber and disperser the analytical results from samples drawn during the first 5-10 minutes showed consistently an increase and then levelled off and became constant. There was no reason to suspect other causes than an initial wall adsorption and final adsorption equilibrium to cause this phenomenon. In order to confirm this suspicion, crude experiments with fruit flies were made. The notebook entries are as follows:

March 11, 1953

Chamber in operation from 9 a.m. until 10 a.m. with a concentration of 270 mmg./l. Exposure duct intermittently connected to disperser duct for periods of 2-3 minutes for sample taking. Disperser removed at 10 a.m., exhaust motor continued to run until 3:40 p.m. After shutting the exhaust motor off, 2 mesh wire cages (one inch in diameter, 4 inches high) with 20 fruit flies in each were placed in the exposure compartment. The exposure valve was opened and left in this position. At 5:30 p.m. all flies were dead. No observation in between.

March 12, 1953

At 9 a.m. the exhaust motor of the chamber was switched on with the exposure compartment closed against the disperser duct (disperser not operating). After several minutes of this flushing out process, the exhaust fan was stopped. The exposure valve was opened and two fresh cages with fruit flies were placed into the chamber at 9:20 a.m. At 9:30 a.m. the flies were less active. At 9:40 all were dead.

March 13, 1953.

Chamber operated from 10 a.m. to 11 a.m. with 240 mmg./l. Sampling operations as on Mar. 11. Removal of disperser at 11:10 a.m. Exhaust motor continued to run with the exposure compartment connected to fresh air. At 1:30 p.m. the valve was put in exposure position and one cage with fruit flies was placed in the center of the exposure compartment and one as closely as possible into one corner. During the next 2 hours the flies seemed to lose some of their activity as observed through the plexiglass window, but none died. The exhaust motor was stopped at 3:30 p.m. without changing the valve position. At 3:55 p.m. all fruit flies in the corner cage were dead, while several were still alive in the center cage. At 4 p.m. all fruit flies were dead.

These experiments confirmed the existence of strong wall adsorption. They indicate that even after many hours of flushing the chamber with 3000 liters of fresh air per minute, there is enough active agent left adsorbed on the wall (probably absorbed by the stainless steel powder coating) to kill fruit flies in a few minutes. The fruit flies do not die if a stream of fresh air passes through the chamber because the agent diffusing away from the wall becomes too diluted, but they die rapidly after stopping the air flow because a higher concentration can build up throughout the chamber.

It was therefore decided to run the chamber previous to the first exposure every morning for about five minutes with the lever in exposure position in order to establish adsorption equilibrium on the walls. This procedure improved the stability of concentration. The lever was also left open between exposures, except for the few seconds needed to insert and remove the animals. This was the reason for not allowing for an animal adaptation period prior to exposure.

APPENDIX V

by

H. J. Trurnit

A report from Ottawa just received (Annual Report on the Progress of Defense Medical Research, for the calendar year 1951, printed February 1953) contains GB toxicity data for maccacus rhesus monkeys. Twelve monkeys were exposed to GB vapor singly and fourteen monkeys, two at a time, both groups for ten seconds. The approximate LCt_{50}'s were obtained by plotting dosage (Ct) against percentage mortality. The approximate LCt_{50} for the first group was -20, for the second group it was 30.

These data agree very well with those shown in Figure 10 of this report.

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SUBMITTED:

H. J. TRURNIT*
Toxicology Division

P. D. BALES*
Applied Physiology Branch

E. J. ESPOSITO*
Toxicology Division

P. HOROWITZ*
Toxicology Division

*Authors

APPROVAL RECOMMENDED:

WILLIAM H. CHAMBERS
Chief, Physiology Division

S. D. SILVER
Chief, Toxicology Division

APPROVED:

DAVID B. DILL
Scientific Director
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NORMAN W. ELTON
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