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CHEMICAL WARFARE LABORATORIES
TECHNICAL REPORT

CWLR 2031

THE MEDIAN LETHAL CONCENTRATION OF
DIBORANE VAPOR FOR RATS AND MICE (U)

by

Lorraine H. Lawson
Keith H. Jacobson

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POR FOR RATS AND MICE (U) - Lorraine H. Lawson, Keith H.
Jacobson

CWLR 2031, 3 Mar 56, 13 pp - illus - tables (UNCLASSIFIED)
Project 4-61-14-002

Median lethal concentrations for 4-hr. exposures of rodents to diborane were determined to facilitate comparison of the acute toxicities of various boron compounds. These LC50 values were 80 p.p.m. for 5-mo.-old Chemical Warfare Laboratories Colony rats, 40 p.p.m. for 2-mo.-old Edgewood Breeding Farms rats, and 29 p.p.m. for Carworth Farms mice. Evidence from an additional experiment with the two strains of rats suggests that the difference in LC50 values for the rats results in greater measure from the age or weight difference than from the strain difference. Flies were also exposed to diborane, and results from these exposures show that flies are not useful as diborane detectors.

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1. Diborane, LC50's for rats and mice

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Chemical Corps Research and Development Command
CHEMICAL WARFARE LABORATORIES
Army Chemical Center, Maryland

Chemical Warfare Laboratories Report No. 2031

Directorate of Medical Research

THE MEDIAN LETHAL CONCENTRATION OF DIBORANE VAPOR FOR RATS AND MICE (U)

by

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Toxicology Division

Chemical Warfare Laboratories
Report No.2031

APPROVED:

THE MEDIAN LETHAL CONCENTRATION OF
DIBORANE VAPOR FOR RATS AND MICE (U)



ALBERT R. DREISBACH
Colonel, Medical Corps
Director of Medical Research

Project No.: 4-61-14-002
Notebook No.: 181

Date Started: 21 December 1953
Date Completed: 5 April 1954



S. D. SILVER
Deputy Commander for
Scientific Activities

Typed: 3 March 56
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THE MEDIAN LETHAL CONCENTRATION OF DIBORANE VAPOR FOR RATS AND MICE (U)

ABSTRACT

Median lethal concentrations for 4-hour exposures of rodents to diborane were determined to facilitate comparison of the acute toxicities of various boron compounds. These LC50 values were 80 ppm for 5-month-old Chemical Warfare Laboratories Colony rats, 40 ppm for 2-month-old Edgewood Breeding Farms rats, and 29 ppm for Carworth Farms mice. Evidence from an additional experiment with the two strains of rats suggests that the difference in LC50 values for the rats results in greater measure from the age or weight difference than from the strain difference.

Flies were also exposed to diborane, and results from these exposures show that flies are not useful as diborane detectors.

Chemical Warfare Laboratories Report No. 2031

THE MEDIAN LETHAL CONCENTRATION OF DIBORANE VAPOR FOR RATS AND MICE (U)

I. INTRODUCTION.

The increasing interest in the toxicology of boron compounds has indicated a need for comparative estimates of their toxicities. Since diborane is one of the best known of these compounds, it seems a logical standard for toxicological comparison of the respiratory irritant class of boranes. The object of the study described herein was to determine the median lethal concentration of diborane to several species and strains of laboratory animals in order to facilitate comparison with similar acting compounds. A secondary object that developed during the study was to investigate the usefulness of flies in the detection of diborane. This work was authorized by the Chief Chemical Officer under Project 4-61-14-002, Health Hazards of Military Chemicals, Test Program No. 232, Cml C Research and Development Program for fiscal year 1954.

This work is an extension, made possible by further knowledge about and experience in handling diborane, of the investigations of Comstock, Feinsilver, Lawson, and Oberst (1), who made the first systematic study of the toxicity of diborane. They found it to be a highly toxic gas, causing pulmonary edema in exposed animals.

II. EXPERIMENTAL.

A. Procedure.

A cylinder containing 6 oz. of diborane, obtained from the Mathieson Chemical Company, was used. The cylinder was placed in a can packed with dry ice to facilitate control of gas flow. The diborane was passed into a 400-liter, dynamic chamber at rates ranging from 3.5 to 7.7 ml./min.; it was mixed with chamber air flowing at a rate of 200 l./min. The chamber air was sampled by drawing 30 l. through a series of two Edgewood-type bubblers containing ethyl cellosolve distilled over K_2CO_3 at 134-135°C. and redistilled over CaO at 134-136°C. (2). Boric acid formed from hydrolysis was determined titrimetrically with 0.02 N sodium hydroxide, phenolphthalein being used as an indicator (1).

Male white rats, from the Edgewood Breeding Farms and from the Chemical Warfare Laboratories Colony, and female white mice from the Carworth Farms were used in this investigation. All exposures were for single 4-hour periods. Median lethal concentrations (LC50's) were estimated by the Bliss-Finney method (3). Ten rodents were used at each concentration level; all deaths occurring during exposure and within 14 days thereafter were counted in calculating LC50 values. Additional rodents were exposed and periodically sacrificed in a study of pathological changes. Tissues taken for microscopic study were from lungs, esophagus, trachea, heart, liver, and spleen.

B. Results.

The mortality observed in male rats (2-month-old) from Edgewood Breeding Farms during exposure and for 14 days thereafter is listed in Table I. The LC50 value for these rodents was 40 ppm. All deaths occurred within 2 days after exposure except for one rat that had been exposed to 43 ppm; this particular rat died on the 14th day. The mortality in the male rats (5-month-old) from the Chemical Warfare Laboratories Colony is shown in Table I. The LC50 was 80 ppm. The mortality observed in female mice is listed in Table I. The LC50 was 29 ppm. A statistical summary of these mortality data in rodents is shown in Table II.

All severely poisoned rodents showed labored breathing and froth from the nose. These signs were not seen in less severely poisoned animals except just prior to death.

The lungs of the rats autopsied on the day of exposure were edematous.* Rats sacrificed 14 days after exposure did not show this condition. This edema was evident both macroscopically and microscopically. Occasionally it was confined to peribronchial and perivascular lymphatic spaces.

In view of the difference in LC50 values noted in the two different strains of rats, it was of interest to see if this could be verified. Some of the Edgewood Breeding Farms rats left over from the previous work were allowed to age to 5 months. Additional Edgewood Breeding Farms rats, 2 months old, and Chemical Warfare Laboratories Colony rats, 5 months old, were procured. All animals were exposed simultaneously for 4 hours to 53 ppm. The results are shown in Table III. Assuming a slope function (4) of 1.3, the value found in the dose-response curves cited above, the following LC50 values may be estimated from these results: Edgewood Breeding Farms rats, 2 months old, 42 ppm; Edgewood Breeding Farms rats, 5 months old, 65 ppm; Chemical Warfare Laboratories Colony rats, 5 months old, 74 ppm.

We were also interested in the possible use of flies in the detection of diborane vapor. A group of 60 male and 60 female Drosophila (fruit flies)** were exposed 4 hours to 62 ppm. None were knocked down or killed during exposure; however, all but one died overnight. Control flies also died overnight, and deaths in test and control groups were believed due to desiccation. In another exposure, 60 male and 60 female Drosophila, and 60 male and 60 female Musca (houseflies) were exposed 4 hours to 32 ppm. None were knocked down or killed, and all were alive 24 hours later, as were the controls. It appears from this that flies are less susceptible than rats to the effects of diborane vapor, and are not useful as diborane detectors.

* Pathological studies were made by Captain Max Colton and Mr. Paul Yevich, Pathology Branch, Directorate of Medical Research.

** Flies were furnished and counted by Dr. Fred M. Snyder, Entomology Branch, Directorate of Medical Research. The species used were D. melanogaster (Meigen) and M. domestica L.

III. DISCUSSION.

The previously reported pulmonary irritant properties of diborane (1) have been confirmed in this study.

The purpose of this investigation was to determine the median lethal concentration of diborane for comparison with similar acting derivatives of diborane. Feinsilver et al. (2) reported an LC50 for male Pied Piper Farm rats, 2½-3 months old, exposed 4 hours to dimethylaminodiborane vapor, to be 86 ppm. The LC50 for male mice of the same age, also exposed for 4 hours, was 63 ppm. Comparing mice and rats of one strain or sex with those of another strain or sex has its pitfalls; nevertheless, we believe that we can make useful comparisons. Our experience suggests that Pied Piper Farm rats are similar to Edgewood Breeding Farms rats in sensitivity to respiratory irritants. If we are to make comparisons, we should therefore prefer to compare the LC50 of dimethylaminodiborane with the LC50 we found for 2-month-old Edgewood Breeding Farms rats; this figure was found to be 40 ppm for 4 hours' exposure. Thus, the potency ratio (i.e., LC50 of dimethylaminodiborane divided by the LC50 of diborane) is 2.2 for rats and 2.1 for mice. From this reasoning and subsequent calculations, we conclude that dimethylaminodiborane is about half as toxic as diborane. Of course, different potency ratios would be found if LC50's were compared in terms of mg./m.³. We chose to compare these values in terms of ppm because ratios of concentrations in ppm are ratios of numbers of molecules. Therefore, it takes twice as many molecules of dimethylaminodiborane as molecules of diborane to cause death in rats and mice.

The two estimates of the LC50 of diborane for 2-month-old Edgewood Breeding Farms rats (40 and 42) are significantly different at $P < 2\%$ from the two estimates of 5-month-old Chemical Warfare Laboratories Colony rats (80 and 74). The estimated LC50 of 65 for the 5-month-old Edgewood Breeding Farms rats is significantly different from the values of 40 and 42, but not from the value of 74. From this it follows that the quantitative response of 2-month-old Edgewood Breeding Farms is significantly different from that of 5-month-old rats of the same strain and from the 5-month-old Chemical Warfare Laboratories Colony rats. It appears that the age difference is responsible in greater measure than the strain difference for this difference in response. What we attribute to age difference may, of course, be due to weight difference, since among these rats, weight and age changes tended to be parallel.

Subsequent to the completion of our work, concentration-response curves for various compounds were recalculated using a 7-day post-exposure observation period instead of the standard 14-day period. It was found that there were no significant differences in the LC50 values between the two observation periods; hence, a 7-day period has been adopted as the normal observation period. Since future comparisons of the values given in this report may be made with other statistics based on a 7-day period, the LC50 values reported herein have been calculated for both observation periods. As can be seen from Table II, there are no differences between the curves for the two periods, except for a slight, non-significant difference in the LC50 values and slopes of the two curves for mice.

IV. CONCLUSIONS.

The respiratory irritant properties of diborane are confirmed. The LC50 for 2-month-old rats obtained from the Edgewood Breeding Farms is 40 ppm (45.3 mg./m.³). The LC50 for the 5-month-old rats obtained from the Chemical Warfare Laboratories Colony is 80 ppm (90.6 mg./m.³). The LC50 for female mice is 29 ppm (32.8 mg./m.³). It appears that the age difference is responsible in greater measure than the strain difference for this difference in response.

Flies are not useful as diborane detectors. -

V. ACKNOWLEDGEMENT.

We are indebted to Ira A. DeArmon and G. Stanley Woodson for making statistical calculations of our data.

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Table I

Mortality in Rodents Exposed 4 Hours to Diborane

Species	Concentration		Time of Deaths Days After Exposure						Mortality	
	mg./m. ³	ppm	with- drawal	1	2	3	4-7	8-14	Fraction	%
Two-month-old male Edgewood Breeding Farms rats.	21.1	19	-	-	-	-	-	-	0/10	0
	36.1	32	-	1	1	-	-	-	2/10	20
	42.5	38	-	4	-	-	-	-	4/10	40
	48.2	43	-	4	2	-	-	1	7/10	70
	58.9	52	-	6	2	-	-	-	8/10	80
	70.0	62	-	9	1	-	-	-	10/10	100
Five-month-old male Chemical Warfare Laboratories Colony rats.	50.2	44	-	-	-	-	-	-	0/10	0
	68.9	61	1	1	-	-	-	-	2/10	20
	83.4	74	1	2	-	-	-	-	3/10	30
	89.8	79	1	-	3	-	-	-	4/10	40
	93.7	83	2	4	-	-	-	-	6/10	60
	99.5	88	1	3	2	1	-	-	7/10	70
Three-month-old female Carworth Farms mice.	25.0	22	-	-	-	-	-	1	1/10	10
	27.5	24	-	-	1	-	-	-	1/10	10
	32.1	28	-	2	-	1	-	3	6/10	60
	37.6	33	-	3	2	1	1	-	7/10	70
	38.1	34	-	1	1	6	-	-	8/10	80
	50.2	44	-	-	8	-	1	-	9/10	90

Table II

Comparison of LC50 Values and Other Statistics for
4-Hour Exposure of Rodents to Diborane*

Species	Post-Exposure Observation Period	LC50	19/20 Confidence Limits	Slope	Standard Error of Slope
	days		ppm		
Male Edgewood Breeding Farms Rats (2 mos. old)	7	40	36-45	9.6	2.4
	14	40	35-45	9.7	2.4
Male Chemical Warfare Laboratories Colony Rats (5 mos. old)	7	80	73-93	9.4	3.1
	14	80	73-93	9.4	3.1
Female Carworth Farms Mice (2½-3 mos. old)	7	31	28-34	12.0	2.6
	14	29	27-32	9.6	2.2

* Subsequent to the completion of this work the post-exposure observation was changed from a 14-day period to a 7-day period. The above statistics were therefore calculated for a 7-day period as well as the 14-day period.

Table III

Comparison of Mortality of Several Strains of Rats
Exposed 4 Hours to 53 ppm and Observed 14 Days

Kind of Rats	Time of Death and Mortality %	Estimated LC50
Edgewood Breeding Farms Rats (2 mos. old)	8/10 in 2 days 80%	42
Edgewood Breeding Farms Rats (5 mos. old)	3/14 in 4 days 24.5%	65
Chemical Warfare Laboratories Colony Rats (5 mos. old)	1/10 in 1 day 10%	74

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- 131 -Hercules Powder Company, Hercules Experiment Station, Wilmington, Dela-
ware
- 132-133 -Naval Air Experimental Station, Naval Air Material Center, U. S. Naval
Base, Philadelphia 12, Pennsylvania ATTN: XM-43-VCP
- 134 -Commanding Officer, U. S. Naval Ammunition Depot, Crane, Indiana
- 135 -Commanding Officer, Army Environmental Health Laboratory, Building No.
1235, Army Chemical Center, Maryland
- 136-139 -Commanding Officer, Chemical Warfare Laboratories, Building No. 330,
Army Chemical Center, Maryland ATTN: Technical Library
- 140 -Commanding Officer, Naval Unit, Building No. 65, Army Chemical Center,
Maryland
- 141 -President, Chemical Corps Board, Building No. 483, Army Chemical
Center, Maryland
- 142 -Operations Research Group, Building No. 483, Army Chemical Center,
Maryland
- 143 -CONARC Liaison Office, Building No. 330, Army Chemical Center, Mary-
land
- 144 -Air Force Development Field Representative, Building No. 330, Army
Chemical Center, Maryland
- 145-150 -Canadian Army Technical Representative, Building No. 330, Army Chemical
Center, Maryland
- 151-156 -British Liaison Officer, Building No. 330, Army Chemical Center, Mary-
land
- 157 -Executive Director, Coordinating Committee on Biological-Chemical
Warfare, Office of Assistant Secretary of Defense, Research and
Development, Room 3D 1050, The Pentagon, Washington 25, D.C. ATTN:
Chief, Library Branch



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

JUN 20 2016

RDCB-DSR-S

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

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

SUBJECT: 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 following documents:

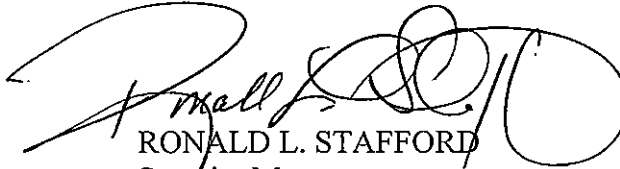
a. Comstock, C.C.; Oberst, F.W.; Lawson, L.; Ozburn, E.E. *The Median Detectable Concentration of Diborane, Pentaborane and Decaborane by Odor for Man*; Medical Laboratories Research Report No. 206; Chemical Corps Medical Laboratories: Army Chemical Center, MD, 1953, Unclassified, Distribution C. **CBRNIAC-CB-111906**

b. Comstock, C.C.; Feinsilver, L.; Lawson, L.H.; Oberst, F.W. *Inhalation Toxicity of Diborane in Dogs, Rats, and Guinea Pigs*; Medical Laboratories Research Report No. 258; Chemical Corps Medical Laboratories: Army Chemical Center, MD, 1954, Unclassified, Distribution C. **ADB032228 CBRNIAC-CB-113261**

c. Lawson, L.H.; Jacobson, K.H. *The Median Lethal Concentration of Diborane Vapor for Rats and Mice*; CWLR 2031; Chemical Warfare Laboratories: Army Chemical Center, MD, 1956, Unclassified, Distribution C. **AD0099186 CBRNIAC-CB-116024**

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

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


RONALD L. STAFFORD
Security Manager