

UNCLASSIFIED

AD NUMBER

AD243462

LIMITATION CHANGES

TO:

Approved for public release; distribution is unlimited.

FROM:

**Distribution authorized to U.S. Gov't. agencies and their contractors;
Administrative/Operational Use; AUG 1960. Other requests shall be referred to Army Chemical Research and Development Laboratories, Edgewood Arsenal, MD.**

AUTHORITY

ECBC memo dtd 6 Jan 2017

THIS PAGE IS UNCLASSIFIED

UNCLASSIFIED

AD 243 462

*Reproduced
by the*

ARMED SERVICES TECHNICAL INFORMATION AGENCY
ARLINGTON HALL STATION
ARLINGTON 12, VIRGINIA



UNCLASSIFIED

NOTICE: When government or other drawings, specifications or other data are used for any purpose other than in connection with a definitely related government procurement operation, the U. S. Government thereby incurs no responsibility, nor any obligation whatsoever; and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use or sell any patented invention that may in any way be related thereto.

ASTIA
NO 243462

U. S. Army
Chemical Research and Development Laboratories
Technical Report

CRDLR 3023

Lethality of VX in Rats at High and Low Temperature

by

H. M. Frankel
J. S. Wiles

August 1960



ARMY CHEMICAL CENTER, MD

FOR OFFICIAL USE ONLY

August 1960

CRDLR 3023

LETHALITY OF VX IN RATS AT HIGH AND LOW TEMPERATURE

by

H. M. Frankel
J. S. Wiles

Physiology and Toxicology Divisions

Recommending Approval:

Douglas Lindsey
DOUGLAS LINDSEY
Lt. Col., MC
Director of Medical Research

Approved:



S. D. SILVER
Deputy Commander for
Scientific Activities

U. S. ARMY
Chemical Corps Research and Development Command
CHEMICAL RESEARCH AND DEVELOPMENT LABORATORIES
Army Chemical Center, Maryland

FOR OFFICIAL USE ONLY

FOREWORD

This work was conducted under Task 4C08-02-023-01, Basic and Applied Physiology (U), and Task 4C08-02-023-04, Toxicology of CW Agents (U). The work was started in January 1959 and completed in July 1959. The experimental data are recorded in notebook MN-1127.

Notice

Reproduction of this document in whole or part is prohibited except with permission of the issuing office; however, ASTIA is authorized to reproduce the document for U. S. Governmental purposes.

Disposition

When this document has served its purpose, DESTROY it. DO NOT return to U. S. Army Chemical Research and Development Laboratories.

FOR OFFICIAL USE ONLY

DIGEST

The object of this research was to compare the lethality of VX injected intravenously and applied percutaneously at low, normal, and high environmental temperatures.

The conclusions reached are as follows:

1. Acute exposure of rats at 2° or 34°C increased the toxicity of VX administered intravenously or percutaneously when compared with exposure at 26°C.
2. The effect of temperature on lethality of VX is more marked with percutaneous application than with intravenous injection.

FOR OFFICIAL USE ONLY

LETHALITY OF VX IN RATS AT HIGH AND LOW TEMPERATURE

I. INTRODUCTION.

Acute exposure to high or low environmental temperature modifies the toxicity of anticholinesterase compounds in animals. Oberst et al.¹ have shown that at a temperature ranging from 32° to 38°C, the $\overline{\text{LCt50}}$ for GB on the skin of monkeys was 59% less than when the animals were exposed at a temperature ranging from 21° to 27°C. These findings were supported by the work of Frankel and Craig.² McPhail³ has reported that the LCt50 of GB inhaled by rats exposed at from -18°C to 0°C was from 12% to 86% less than the value obtained at from 13° to 16°C. However, McPhail and Buckholz⁴ showed that the LD50 of GB on the skin of pigs was 50% greater during exposure at 3°C than at 16°C. Similarly, in monkeys the LD50 on the skin was 50% greater at 3°C than at from 23° to 28°C.

Marzulli and Callahan⁵ were of the opinion, from blood cholinesterase changes in goats, that GB was more rapidly absorbed during the spring and summer seasons than in the winter. It has been reported⁶ that the Lt50 for VX on the skin of cats and rabbits was shorter at 32°C and longer at 2°C than at 26°C. These effects were modified by acclimation and the site of application.⁷

That penetration of the skin is not the only factor in these changes in percutaneous toxicity is seen from the following. Exposure to high or low environmental temperature will decrease the intravenous LD50 of GB in rats.⁸ Streicher⁹ demonstrated a decreased LD50 for DFP injected intraperitoneally in mice exposed at 4°C. Also, 30 μl of GB/kg injected intramuscularly killed more monkeys exposed at 38°C than at 25°C.² Baetjer and Smith¹⁰ showed, in mice injected intraperitoneally and intravenously, that exposure at either 16° or 36°C increased the toxicity of parathion over the toxicity at room temperature.

The reason for the existence of different tolerances at various environmental temperatures is not known. It has been suggested that the rate and efficiency of absorption may be factors. Ainsworth¹¹ applied GB containing radioactive phosphorus to rabbit skin and demonstrated that the concentration of labeled phosphorus in the body was inversely related to the skin temperature. The present study was designed to isolate the contribution of penetration through skin from the other factors that produce changes in toxicity with temperature.

FOR OFFICIAL USE ONLY

II. INVESTIGATIONAL PROCEDURES.

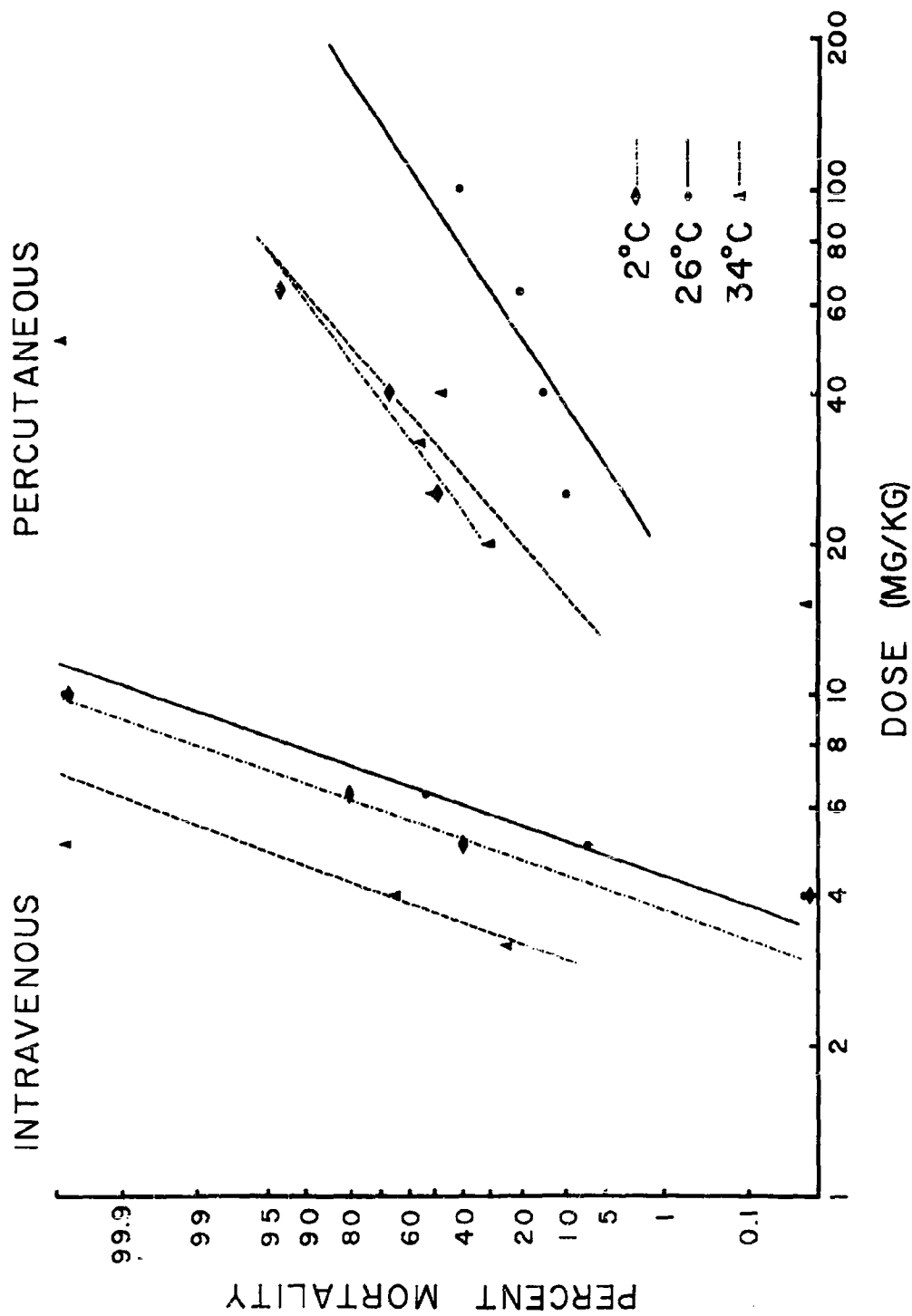
Male albino rats, weighing from 167 to 380 gm, were exposed to controlled temperatures of 2°, 26°, or 34°C for 20 hours before and for 24 hours after application of VX. They were housed in community cages 24 by 18 by 18 in. containing 10 to 12 animals. The agent was injected either directly into the femoral vein or applied as a drop to the surface of the skin on a clipped area in the middle of the back. The animals were selected at random for the dosage and temperature treatment they were to receive. Rats that were given agent on the back were placed in individual compartments 4 by 8 by 4 in. to decrease the opportunity of their licking the agent. Dilutions of the agent were made immediately before use. The first two dilutions were in propylene glycol and the final dilution for intravenous injection was in isotonic saline. The final dilution for percutaneous application was in isopropanol. Dosage was expressed in microliters of undiluted agent, sp gr 1.004.

All animals were injected at room temperature and were returned to the controlled temperature within a few minutes after injection. Application of the agent percutaneously was carried out at the controlled temperature. Agent was given to animals at all three temperatures during the same test period. Deaths were recorded during the following 24 hours. Regression slope and LD50 were calculated by the Bliss method.¹² A "p" value of 0.05 was used to test significance.

III. RESULTS.

Exposure to 2° or 34°C tended to increase the toxicity of a given dose compared with exposure at 26°C when the VX was administered intravenously or percutaneously (see figure). The intravenous LD50's at 2° and 34°C were 86% and 59%, respectively, of the doses required at 26°C. The percutaneous doses were 30% and 35% for 2° and 34°C, respectively, of the dose required at 26°C, as shown in table. The percutaneous traversal of VX, that is, the ratio of $\frac{\text{LD50, intravenous}}{\text{LD50, percutaneous}}$ has been used by Marzulli¹³ for a measure of agent effectiveness. In the present study this ratio was 0.196 at 2°C, 0.070 at 26°C, and 0.116 at 34°C.

FOR OFFICIAL USE ONLY



FIGURE

MORTALITY WITH DOSE OF VX ADMINISTERED INTRAVENOUSLY OR PERCUTANEOUSLY
AT 2°, 26°, AND 34°C IN MALE RATS.

TABLE

LETHALITY OF VX IN RATS AT VARIOUS TEMPERATURES

Route of administration	Environmental temperature	LD50	95% Confidence limits	Number of rats	Regression slope
	°C	μl/kg			
Intravenous	2	5.4	4.9-5.9	35	14.55
Intravenous	26	6.3	5.8-7.4	45	13.86
Intravenous	34	3.7	3.3-4.1	22	15.23
Percutaneous	2	27.6	20.0-38.1	45	3.71
Percutaneous	26	91.7	70.4-119.6	65	3.34
Percutaneous	34	31.9	26.5-38.2	89	4.42

IV. DISCUSSION.

At 26°C, 91.7 μl/kg of VX must be placed on the skin of rats to produce the same effect as 6.3 μl/kg injected into the blood stream. The difference, or 85.4 μl/kg, represents agent that was lost or inactivated somewhere along the line from the surface of the skin to the blood stream. Either this difference or the ratio between the percutaneous and the intravenous doses may provide a measure of the resistance or penetrability of the skin or of efficiency of absorption. From the percutaneous traversal ratios described in the results it appears that agent is more effectively absorbed through the skin of rats exposed at 2° and 34°C than at 26°C.

The mechanism responsible for the change in efficiency is not clear. It cannot be circulation alone, because in the cold circulation in the skin tends to decrease. It may be a combination of factors, such as, the time the agent is in contact with the skin and circulation to the area. Thus, in the cold, time on the surface may increase and circulation decline; in the heat, time may decrease and circulation to the area increase. In this report it has been the authors' observation that time to death was longer in the cold than at the highest temperature. A measurement of circulation in the area at the time the agent is in contact with the skin would help in the interpretation of these results.

FOR OFFICIAL USE ONLY

V. CONCLUSIONS.

The conclusions reached are as follows:

1. Acute exposure of rats at 2° or 34°C increased the toxicity of VX administered intravenously or percutaneously when compared with exposure at 26°C.

2. The effect of temperature on lethality of VX is more marked with percutaneous application than with intravenous injection.

FOR OFFICIAL USE ONLY

LITERATURE CITED

1. Oberst, F. W., McGrath, F. P., and DeArmon, I. A. MLRR 147. The Protective Action of Standard Clothing Material Against Percutaneous Exposure of Monkeys to GB Vapor. November 1952. (UNCLASSIFIED Report).
2. Frankel, H. M., and Craig, F. N. CWLR 2032. Physiological Actions of GB in a Warm Environment. June 1956. (UNCLASSIFIED Report).
3. McPhail, M. K. 3rd Symposium, pp. 255-269. Defense Research Board, Canada. December 1951. (SECRET Report).
4. McPhail, M. K., de Candole, C. A., and Stewart, W. C. Defense Research No. 131, Vol 1, p. 120. July 1958. (SECRET Report).
5. Marzulli, F. N., and Callahan, J. F. MLRR 391. Studies on the Mechanism of Skin Penetration and Absorption of GB in Percutaneously Poisoned Goats, as Determined by Measurements of Red Blood Cell and Cerebral Cortex Cholinesterase. August 1955. (UNCLASSIFIED Report).
6. Thirteenth Tripartite Conference on Toxicological Warfare. U. S. Progress Report on Medical Aspects. July 1958. (SECRET Report).
7. LeBlanc, J., and Rosenberg, F. CWLR 2192. Effect of Cold Acclimatization on the Physiological Action of a Chemical Agent in Rats. November 1957. (CONFIDENTIAL Report).
8. Frankel, H. M., and Blevins, W. V. CWLR 2348. Effect of Environmental Temperature on Toxicity of GB Intravenously Administered. January 1960. (UNCLASSIFIED Report).
9. Streicher, E. Toxicity of Colchicine, Di-isopropyl Fluorophosphate, Intocostrin, and Potassium Cyanide in Mice at 4°C. Proc. Soc. Exptl. Biol. Med. 76, 536-538 (1951).
10. Baetjer, A. M., and Smith, R. Effect of Environmental Temperature on Reaction of Mice to Parathion, an Anticholinesterase Agent. Am. J. Physiol. 186, 39-46 (1956).
11. Ainsworth, M. PTP 426. Some Factors Influencing the Percutaneous Toxicity of GB. May 1954. (UNCLASSIFIED Report).

FOR OFFICIAL USE ONLY

12. Bliss, C. Y. Statistics of Bioassay. pp. 536-547. Academic Press, N.Y. 1952.

13. Marzulli, F. N. MLSR 75. A Comparison of Toxic Properties of the V Agents With GB. December 1955. (SECRET Report).

FOR OFFICIAL USE ONLY



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

JAN 06 2017

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 (DTIC), 8725 John J. Kingman Road, Ft Belvoir, VA 22060-6218

SUBJECT: 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 attached listed documents:
2. The listed documents have current distribution statements or classifications which limit their release. ECBC Subject Matter Experts have reviewed the documents and deemed them suitable for the change in distribution to read "Approved for public release; distribution unlimited."
3. The point of contact is Adana Eilo, ECBC Security Specialist, (410) 436-2063 or adana.l.eilo.civ@mail.mil.

Encl


RONALD L. STAFFORD
Security Manager

ECBC Documents for Downgrading/Change in Distribution

1. Callahan, J.F. *The Relation between Skin Thickness and the Penetration Rate of VX through Skin*. In *Research Program of the Field Toxicology Branch*; CRDL TM 20-27; Callahan, JF, et al. Eds.; Directorate of Medical Research, U.S. Army Chemical Research and Development Laboratories, U.S. Army Chemical Center: Edgewood Arsenal, MD, 1962; UNCLASSIFIED Report. **CBRNIAC-CB-118810 Dist. E.**
Recommended for public release.
2. Callahan, J.F.; Cresthull, P.; Christensen, M.K.; Crook, J.W.; Wiles J.S.; Owens, E.; Hart, J.; Worden, F.X. *Intravenous Toxicity of VX in Marzulli et al. Biological Studies on VX during Fiscal Year 1958*; CWL Special Publication 2-18; U.S. Army Chemical Warfare Laboratories: U.S. Army Chemical Center, 1959; UNCLASSIFIED Report **AD0313760 Dist. C.**
Recommended for public release.
3. Frankel, H.M.; Wiles, J.S. *Lethality of VX in Rats at High and Low Temperatures*; CRDLR-3023; U.S. Army Chemical Research, and Development Laboratories: Edgewood Arsenal, MD, 1960; UNCLASSIFIED Report **AD0243462 Dist. C.**
Recommended for public release.
4. Marzulli, F.N. *A Comparison of Toxic Properties of the V Agents with GB*; MLSR-75; U.S. Army Chemical Corp Medical Laboratories: U.S. Army Chemical Center, MD, 1955; UNCLASSIFIED Report **AD0090916 Dist. C.**
Recommended for public release.
5. Reutter-Christy, S.A.; Sommerville, D.R.; Edward M. Jakubowski; Christopher E. Whalley; Bernard J. Benton; Stanley W. Hulet; Paul A. Dabisch; Ronald A. Evans; Jeffrey M. McGuire; Charles L. Crouse; R Christopher E. Byers; James H. Manthei; Ruth W. Moretz; Jeffry S. Forster; Bernardita I. Gaviola; David C. Burnett; William T. Muse; Kathy L. Matson; Robert J. Mioduszewski; Sandra A. Thomson; Julie A. Renner; Allison L. Totura; Edward J. Emm; Stephen R. Channel; Tsung-Ming Shih; Lucille A. Lumley; John O'Donnell; Theresa Ward; Bountieng Somsamayvong; Christopher Robison; Susan Schulz; Kelly L. Ault; Edward D. Clarkson; Raymond F. Genovese; John L. Oubre; Patrick J. Fleming. *Chemical Warfare Agent Operational Exposure Hazard Assessment Research: FY07 Report and Analysis*; ECBC-TR-784; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 2010a. **ADB370363 Dist. C. Recommended for public release.**
6. Reutter, Sharon A.; Moretz, Ruth W.; Murray, Michele M.; Sommerville, Douglas R., *Review of Toxicological Data Regarding Contact Hazards of Chemical Agents*, ECBC-TR-514; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 2006; UNCLASSIFIED Report **ADB321921 Dist. C. Recommended for public release.**
7. Sim, V.M. *Variability of Different Intact Human-Skin Sites to the Penetration of VX*; CRDLR-3122; Chemical Research and Development Laboratories: Edgewood Arsenal, MD, 1962 **AD0271163 Dist. C. Export Control Recommended for public release.**
8. Sim, V.M.; Stubbs, J.E. *VX Percutaneous Studies in Man*; CRDLR-3015; Chemical Research and Development Laboratories: Aberdeen Proving Ground, MD, 1960 **AD0318533 Dist. F. Recommended for public release.**