



U.S. ARMY COMBAT CAPABILITIES DEVELOPMENT COMMAND CHEMICAL BIOLOGICAL CENTER

ABERDEEN PROVING GROUND, MD 21010-5424

CCDC CBC-TR-1638

Median Lethal Dose (LD_{50}) Associated with Intravenous Exposure to VX in Hairless Guinea Pigs

**Linnzi K. M. Wright
Ruth W. Moretz
Julie A. Renner
Jeffry S. Forster**

RESEARCH AND TECHNOLOGY DIRECTORATE

May 2020

Disclaimer

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorizing documents.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 h per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE (DD-MM-YYYY) XX-05-2020		2. REPORT TYPE Final		3. DATES COVERED (From - To) Jun 2018–Jun 2018	
4. TITLE AND SUBTITLE Median Lethal Dose (LD ₅₀) Associated with Intravenous Exposure to VX in Hairless Guinea Pigs				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Wright, Linnzi K. M.; Moretz, Ruth W.; Renner, Julie A.; and Forster, Jeffry S.				5d. PROJECT NUMBER CB10434	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Director, CCDC CBC, ATTN: FCDD-CBR-TO, APG, MD 21010-5424				8. PERFORMING ORGANIZATION REPORT NUMBER CCDC CBC-TR-1638	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) Defense Threat Reduction Agency, 8725 John J. Kingman Road, MSC 6201, Fort Belvoir, VA 22060-6201				10. SPONSOR/MONITOR'S ACRONYM(S) DTRA	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release: distribution unlimited.					
13. SUPPLEMENTARY NOTES U.S. Army Combat Capabilities Development Command Chemical Biological Center (CCDC CBC) was previously known as U.S. Army Edgewood Chemical Biological Center (ECBC).					
14. ABSTRACT: (Limit 200 words) Dermal interstitial fluid, the fluid found in the spaces between skin cells, is a potentially rich reservoir of information about the body's response to nerve agent exposure. Hairless guinea pigs have been selected as the most appropriate animal model for an upcoming study comparing the biomarkers found in interstitial fluid with those in blood from <i>o</i> -ethyl- <i>S</i> -(2-diisopropylaminoethyl) methyl phosphonothiolate (VX)-exposed animals. However, the median lethal dose (LD ₅₀) associated with VX intravenous exposure is unknown in this animal model. Using an up-and-down procedure, the 24 h LD ₅₀ for VX intravenous exposure was determined to be 12.1 µg/kg with a 95% confidence interval of 7.3 to 22.1 µg/kg.					
15. SUBJECT TERMS Hairless guinea pigs Intravenous exposure <i>O</i> -ethyl- <i>S</i> -(2-diisopropylaminoethyl) methyl phosphonothiolate (VX) Median lethal dose (LD ₅₀) Median effective dose (ED ₅₀)					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Renu B. Rastogi
U	U	U	UU	16	19b. TELEPHONE NUMBER (include area code) (410) 436-7545

Blank

PREFACE

The work described in this report was authorized under project number CB10434. The work was started and completed in June 2018. At the time this work was performed, the U.S. Army Combat Capabilities Development Command Chemical Biological Center (CCDC CBC; Aberdeen Proving Ground, MD) was known as the U.S. Army Edgewood Chemical Biological Center (ECBC).

The use of either trade or manufacturers' names in this report does not constitute an official endorsement of any commercial products. This report may not be cited for purposes of advertisement.

This report has been approved for public release.

Acknowledgments

The authors acknowledge the following individuals for their hard work and assistance with the execution of this technical program:

- Kathy Crouse (CCDC CBC) for conducting the solution checks;
- David McGarvey (CCDC CBC) and William Creasy (Leidos, Inc.; Abingdon, MD) for analyzing the purity of VX; and
- Trevor Glaros and Phillip Mach (CCDC CBC) for the opportunity to collaborate with them on this project.

Blank

CONTENTS

	PREFACE	iii
1.	INTRODUCTION	1
2.	METHODS	1
2.1	Animals	1
2.2	Agent	2
2.3	Exposures	2
3.	RESULTS AND DISCUSSION	3
	LITERATURE CITED	5

FIGURES

Latency to onset of toxic signs for hairless guinea pigs intravenously exposed to VX.....	4
---	---

TABLES

1. Ethogram of the Behavioral Toxic Signs Associated with VX Intravenous Exposure for Hairless Guinea Pigs	2
2. Latency to First Toxic Sign and Latency to Death for Hairless Guinea Pigs Intravenously Exposed to VX.....	3

MEDIAN LETHAL DOSE (LD₅₀) ASSOCIATED WITH INTRAVENOUS EXPOSURE TO VX IN HAIRLESS GUINEA PIGS

1. INTRODUCTION

The Institut national de la recherche scientifique (INRS)-Institut Armand-Frappier (IAF; Quebec, Canada) hairless guinea pig is derived from a spontaneous mutation in the Hartley strain of guinea pigs. Unlike most other hairless rodent models, the IAF guinea pigs are euthymic and immunocompetent (Balk, 1987). The anatomy of their skin is more similar to human skin than to the skin of haired guinea pigs (Sueki et al., 2000), and they are one of the few animal models to be predictive of human skin absorption and penetration (Jung and Maibach, 2015). For these reasons, hairless guinea pigs were selected as the most appropriate animal model for an upcoming study to determine the feasibility of dermal interstitial fluid to serve as a reservoir of biomarkers indicative of *o*-ethyl-*S*-(2-diisopropylaminoethyl) methyl phosphonothiolate (VX) exposure. However, the median lethal dose (LD₅₀) associated with VX intravenous exposure is unknown in hairless guinea pigs. In the only other publication describing the effects of VX intravenous exposure in this animal model (van der Schans et al., 2003), the hairless guinea pigs were anesthetized prior to exposure with ketamine, which has beneficial effects against nerve agents (Barbier et al., 2015; Dorandue et al., 2013).

2. METHODS

In conducting this research, the investigators adhered to the current edition of the *Guide for the Care and Use of Laboratory Animals*. This research was also performed in accordance with the requirements of Army Regulations (AR) 70-18 and the Institutional Animal Care and Use Committee (Office of Laboratory Animal Welfare, National Institutes of Health; Bethesda, MD), which oversees the use of laboratory animals by reviewing for approval all research protocols requiring laboratory animals at the U.S. Army Edgewood Chemical Biological Center (ECBC), now known as the U.S. Army Combat Capabilities Development Command Chemical Biological Center (CCDC CBC; Aberdeen Proving Ground, MD).

2.1 Animals

Male IAF hairless guinea pigs (body weight: 300–350 g), surgically implanted with jugular vein catheters connected to PinPorts (Instech Laboratories, Inc.; Plymouth Meeting, PA), were purchased from Charles River Laboratories International, Inc. (Kingston, NY). These hairless guinea pigs were single-housed in a temperature- and humidity-controlled colony room (22 ± 4 °C and 55 ± 15%, respectively). Lights were left on between 0600 and 1800 h. Food and water were provided ad libitum in home cages in which the hairless guinea pigs also had access to enrichment items. The hairless guinea pigs were acclimated to the facility for at least 4 days before exposure to VX.

2.2 Agent

VX ($90.8 \pm 0.8\%$ pure as determined by ^{31}P nuclear magnetic resonance spectroscopy) was synthesized at ECBC. Adjusting for purity, a 1 mg/mL stock solution was prepared in normal saline and stored at $-20\text{ }^{\circ}\text{C}$ for the duration of the experiment. The stock solution was then diluted to the appropriate concentration (10, 25, or 50 $\mu\text{g/mL}$) with normal saline, and these concentrations (9.9, 27.2, and 45.0 $\mu\text{g/mL}$, respectively) were verified before the VX exposures using gas chromatography–mass spectrometry.

2.3 Exposures

The LD_{50} , as well as the median effective dose (ED_{50}) for moderate toxic signs, associated with VX intravenous exposure was determined for hairless guinea pigs using an up-and-down procedure (Dixon and Mood, 1948; Rispin et al., 2002). Fourteen hairless guinea pigs weighing $345 \pm 18\text{ g}$ (average $\pm$ standard deviation) were intravenously exposed one at a time to VX doses ranging from 3.7 to 21.6 $\mu\text{g/kg}$. The maximum injection volume was 0.5 mL/kg, and the catheter was flushed with saline following VX. Toxic signs (Table 1) were continuously monitored for the first 2 h post-exposure, and the onset of each sign was recorded. Lethality was assessed at 24 h post-exposure, and survivors were euthanized with the intravenous administration of a barbiturate euthanasia solution (390 mg/mL sodium pentobarbital).

Table 1. Ethogram of the Behavioral Toxic Signs Associated with VX Intravenous Exposure for Hairless Guinea Pigs

Category	Toxic Sign	Description
Mild	Ataxia	Inability to coordinate muscle movements
	Exophthalmos	Abnormal protrusion of the eyeballs
	Salivation	Secretion of saliva
Moderate	Tearing	Secretion of milky white tears
	Muscle Fasciculation	A small, local, involuntary muscle contraction
	Tremor	Involuntary trembling or quivering; uncontrolled muscle activity
	Subjerking	Uncontrolled jerking of the head
Severe	Collapse	Inability to support body weight but ability to hold head up
	Convulsion	Violent, involuntary muscle contraction or series of muscle contractions
	Gasping	Laborious or convulsive breathing
	Prostrate	Extreme exhaustion and powerlessness coupled with inability to hold head up and loss of righting reflex

3. RESULTS AND DISCUSSION

As shown in Table 2, the no-observed-adverse-effect level for hairless guinea pigs intravenously exposed to VX was 3.7 µg/kg. Hairless guinea pigs intravenously exposed to higher doses of VX exhibited their first toxic sign within 1 to 9 min, and the latency to onset for each toxic sign is shown in Figure. Salivation was the most frequently observed toxic sign, and tearing was the toxic sign with the longest latency to onset. Four hairless guinea pigs died within 24 h of being intravenously exposed to VX.

Table 2. Latency to First Toxic Sign and Latency to Death for Hairless Guinea Pigs Intravenously Exposed to VX

Animal ID	VX Dose (µg/kg)	Latency to First Toxic Sign (min)	Latency to Death (min)
22	4.3	9	N/A
23	5.4	7	N/A
24	8.6	3	N/A
25	10.8	4.5	N/A
26	13.6	4	N/A
27	21.6	1	8
28	17.1	2	25.5
29	13.6	1	18.5
30	10.8	1.5	<1440
31	3.7	N/A	N/A
32	4.3	7	N/A
34	3.7	N/A	N/A
35	4.3	3.5	N/A
36	3.7	N/A	N/A

ID, identification.

N/A, not applicable.

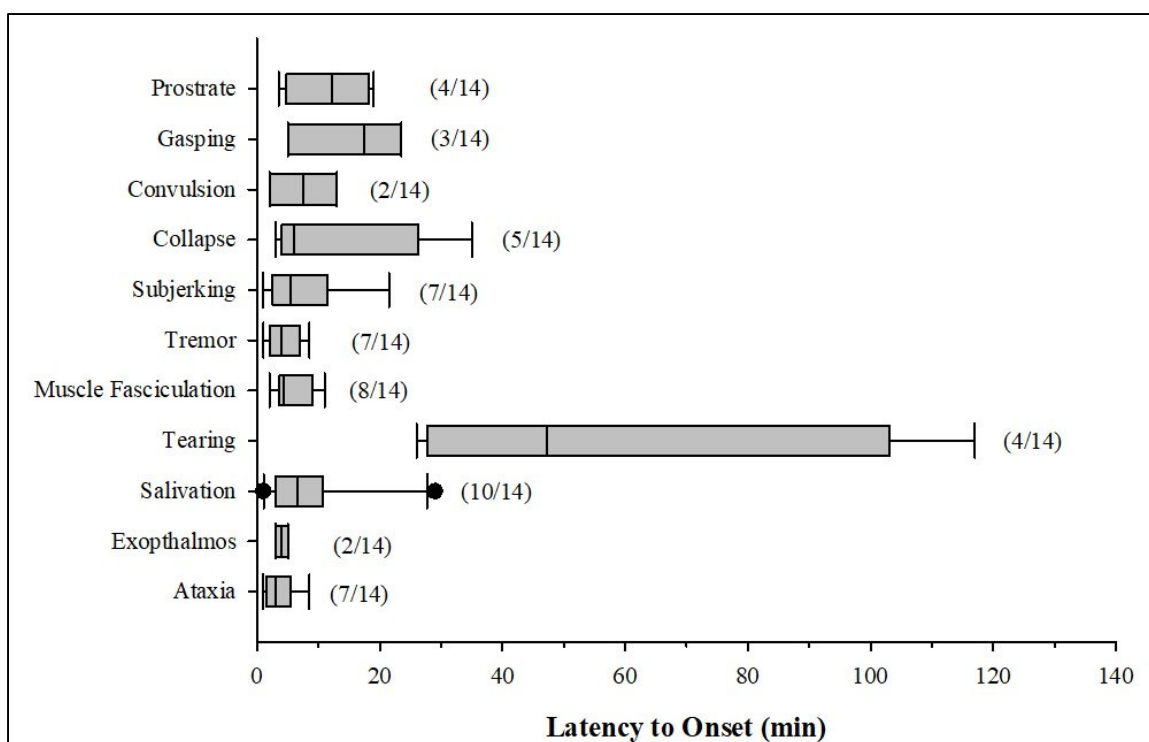


Figure. Latency to onset of toxic signs for hairless guinea pigs intravenously exposed to VX.

Given the small sample size (1–3 hairless guinea pigs per dose) shown in the figure, all of the toxic sign data is combined. The boxes extend from the 25th to the 75th percentiles, and the lines in the middle of the boxes are plotted at the medians. Whiskers are drawn down to the 10th percentile and up to the 90th percentile, whereas points above and below the whiskers are drawn individually. The number of hairless guinea pigs that exhibited each toxic sign compared with the total number of hairless guinea pigs exposed to VX is shown as a ratio in the parenthesis next to each box whisker.

Using the AOT425StatPgm program (U.S. Environmental Protection Agency; Washington, DC) and an assumed sigma of 0.10, the 24 h LD₅₀ for VX intravenous exposure was determined to be 12.1 µg/kg with a 95% confidence interval of 7.3 to 22.1 µg/kg. The ED₅₀ for moderate toxic signs associated with VX intravenous exposure was determined to be 4.1 µg/kg with a 95% confidence interval of 3.7 to 4.3 µg/kg. These values are comparable to those that have previously been reported for haired guinea pigs. Wiles (1974) reported the LD₅₀ for VX intravenous exposure as 7.0 µg/kg. Wright et al. (2017) reported the LD₅₀ as 5.4 µg/kg and the ED₅₀ for moderate toxic signs as 4.0 µg/kg. Thus, hairless guinea pigs are similar to haired guinea pigs in terms of their sensitivity to VX.

LITERATURE CITED

- Balk, M.W. Emerging Models in the USA: Swine, Woodchucks, and the Hairless Guinea Pig. *Prog. Clin. Biol. Res.* **1987**, *229*, 311–326.
- Barbier, L.; Canini, F.; Giroud, C.; Beaup, C.; Foquin, A.; Maury, R.; Denis, J.; Peinnequin, A.; Dorandeu, F. Beneficial Effects of a Ketamine/Atropine Combination in Soman-Poisoned Rats under a Neutral Thermal Environment. *Neurotoxicology* **2015**, *50*, 10–19.
- Dixon, W.J.; Mood, A.M. A Method for Obtaining and Analyzing Sensitivity Data. *J. Am. Stat. Assoc.* **1948**, *43*, 109–126.
- Dorandeu, F.; Barbier, L.; Dhote, F.; Testylier, G.; Carpentier, P. Ketamine Combinations for the Field Treatment of Soman-Induced Self-Sustaining Status Epilepticus. Review of Current Data and Perspectives. *Chem. Biol. Interact.* **2013**, *203*, 154–159.
- Guide for the Care and Use of Laboratory Animals*, 8th ed.; The National Academies Press: Washington, DC, 2011.
- AR 70-18, *Use of Animals in DOD Programs*, Change 1, August 1, 1984.
- Jung, E.C.; Maibach, H.I. Animal Models for Percutaneous Absorption. *J. Appl. Toxicol.* **2015**, *35*, 1–10.
- Rispin, A.; Farrar, D.; Margosches, E.; Gupta, K.; Stitzel, K.; Carr, G.; Greene, M.; Meyer, W.; McCall, D. Alternative Methods for Median Lethal Dose (LD₅₀) Test: The Up-and-Down Procedure for Acute Oral Toxicity. *ILAR J.* **2002**, *43*, 233–243.
- Sueki, H.; Gammal, C.; Kudoh, K.; Kligman, A.M. Hairless Guinea Pig Skin: Anatomical Basis for Studies of Cutaneous Biology. *Eur. J. Dermatol.* **2000**, *10*, 357–364.
- van der Schans, M.J.; Lander, B.J.; van der Wiel, H.; Langenberg, J.P.; Benschop, H.P. Toxicokinetics of the Nerve Agent (±)-VX in Anesthetized and Atropinized Hairless Guinea Pigs and Marmosets after Intravenous and Percutaneous Administration. *Toxicol. Appl. Pharmacol.* **2003**, *191*, 48–62.
- U.S. Environmental Protection Agency. https://www.epa.gov/sites/production/files/2015-06/aot425setup_0.exe (accessed June 2, 2018).
- Wiles, J.S. *(U) Toxicological Evaluation of EA 2223 and EA 5365 by Various Routes of Administration in Several Animal Species*; EB-TR-74021; Department of the Army, Edgewood Arsenal: Aberdeen Proving Ground, MD, 1974; CONFIDENTIAL Report (AD530801).

Wright, L.K.; Forster, J.S.; Moretz, R.W.; Gaviola, B.I.; Renner, J.A.; Kristovich, R.L. *Median Lethal Doses Associated with Intravenous Exposure to the Optically Pure Enantiomers of VX in Guinea Pigs*; ECBC-TR-1437; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 2017; UNCLASSIFIED Report (AD1028936).

DISTRIBUTION LIST

The following individuals and organizations were provided with one Adobe portable document format (pdf) electronic version of this report:

U.S. Combat Capabilities Development
Command Chemical Biological Center
(CCDC CBC)
FCDD-CBR-TO
ATTN: Wright, L.
Hulet, S.

CCDC CBC
Technical Library
FCDD-CBR-L
ATTN: Foppiano, S.
Stein, J.

Defense Threat Reduction Agency
DTRA-RD-IAR
ATTN: Pate, B.

Defense Technical Information Center
ATTN: DTIC OA



U.S. ARMY COMBAT CAPABILITIES DEVELOPMENT COMMAND
CHEMICAL BIOLOGICAL CENTER