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

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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 *o*-ethyl-*S*-(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.

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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).

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1. INTRODUCTION

The Institut national de la recherche scientifique (INRS)-Institut Armand-Frappier (IAF; Quebeq, 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 (LD_{50}) 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 ± 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 °C for the duration of the experiment. The stock solution was then diluted to the appropriate concentration (10, 25, or 50 µg/mL) with normal saline, and these concentrations (9.9, 27.2, and 45.0 µg/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 ± 18 g (average ± standard deviation) were intravenously exposed one at a time to VX doses ranging from 3.7 to 21.6 µg/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.

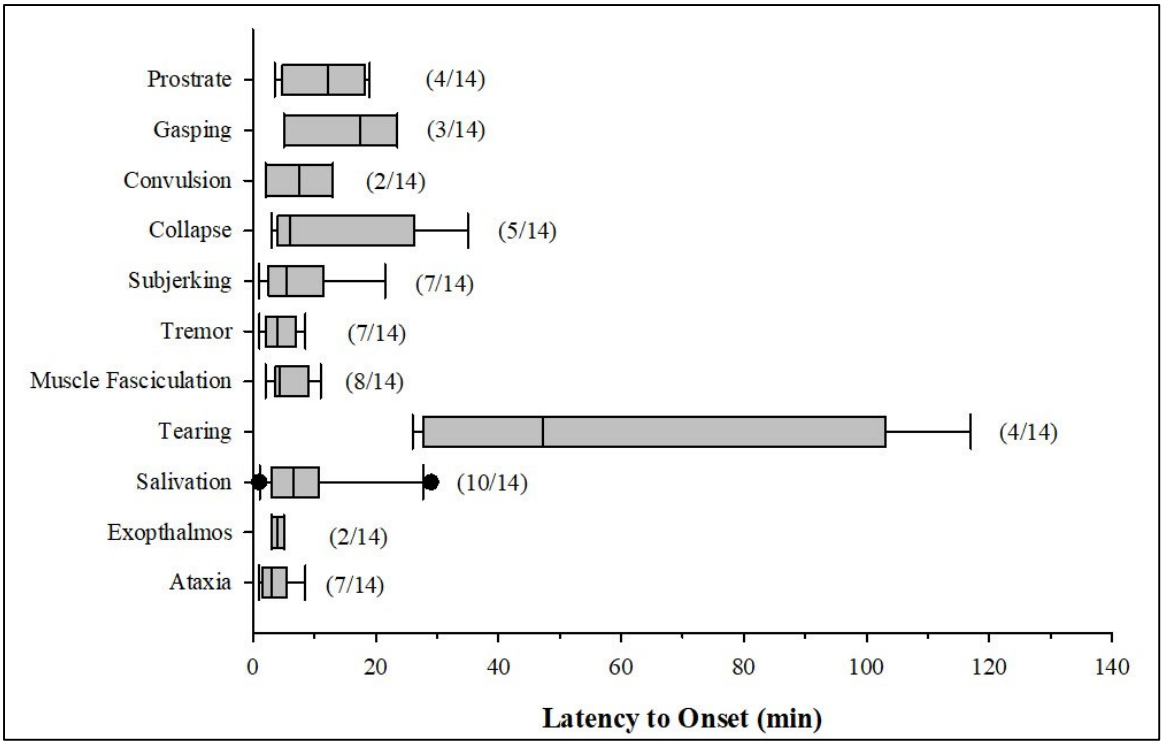

Figure. Latency to onset of toxic signs for hairless guinea pigs intravenously exposed to VX.

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 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. The ED_{50} 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 LD_{50} for VX intravenous exposure as 7.0 µg/kg. Wright et al. (2017) reported the LD_{50} as 5.4 µg/kg and the ED_{50} 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.

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