Estimating Miotic, Severe and Lethal Toxic Effects in Gottingen Minipigs Following Inhalation, Intravenous and Subcutaneous Exposures to VX

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ABSTRACT

Constriction of the pupils (miosis) is often identified as the first noticeable sign of exposure to vapor nerve agents. We have previously identified that in minipigs there is a 30-40 fold difference in vapor dosages of sarin (GB) that elicit miosis vs. those vapor dosages that are potentially lethal. The ratio for miotic vs. lethal vapor dosages ranges from 100-135 fold when cyclo-sarin (GF) is the nerve agent. Due to the extremely low volatility of VX the nerve agent's primary hazard comes from percutaneous absorption. In laboratory settings a combination of high temperatures and airflow can be used to generate vapor VX at stable and measurable concentrations. In a field setting, the generation of VX vapor is less likely, although it is theoretically possible in extreme environments such as the inside of an armored HUMVEE in a desert. In the current studies sexually mature male Gottingen minipigs were exposed to various concentrations of VX vapor in order to determine the lethal (LCT_{50}s) and effective concentrations (ECT_{50}s) for miosis over exposure durations of 10, 60 and 180 minutes. In minipigs VX vapor is less potent than either GB or GF at causing pupil constriction. Additionally, the onset of pupil constriction is delayed in comparison to onset upon exposure to GB or GF. VX vapor was more potent than either GB or GF when lethality was the endpoint. However, unlike with GB or GF vapor exposures, constriction of the pupils was not the definitive first noticeable effect upon a potentially lethal VX vapor exposure. The ratio of ECT_{50} for miosis vs. LCT_{50} values for vapor VX exposures ranges from 3-6 fold. Therefore, the dosages of VX vapor that elicit pupil constriction are much closer to the dosages that result in severe toxic signs or lethality than are seen for either GB or GF vapor exposures.

Male Gottingen minipigs were also exposed to liquid VX via intravenous (IV) or subcutaneous (SC) injections. Signs of nerve agent exposure were classified as lethal, severe, or moderate. Maximum likelihood estimation using a probit model was used to calculate the median effective (severe effects) and lethal doses (LD_{50}) for IV and SC exposures. For intravenously injected VX, the LD_{50} was 11.8 µg/kg (9.7 – 14.5 µg/kg) and the ED_{50} (severe) was 6.6µg/kg (4.8 – 9.0 µg/kg). For subcutaneously injected VX, the LD_{50} was 16.1µg/kg (12.9 – 19.9 µg/kg) and the ED_{50} (severe) was 12.8µg/kg (10.1 – 16.2 µg/kg). VX was more potent than either sarin (GB) or cyclo-sarin (GF) by both routes of exposure.

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1. INTRODUCTION

O-Ethyl S-[2-(diisopropylamino)ethyl] methylphosphonothiolate (VX) is an extremely toxic organophosphorous (OP) nerve agent. Due to the extremely low volatility [1] of VX (0.00063 mm Hg @ 25°C compared to 2.9 mm Hg @ 25°C for sarin) the nerve agent's primary hazard comes from percutaneous absorption. An equivalent dose of liquid VX is considered as substantially more toxic than related nerve agents such as sarin (GB), cyclosarin (GF), tabun (GA) and soman (GD). In a field setting, the generation of VX as a vapor is less likely, although it is theoretically possible in extreme environments such as the inside of an armored HUMVEE in a desert. In laboratory settings a combination of high temperatures and airflow can be used to generate vapor VX at stable and measurable concentrations. Recently VX vapor exposures were performed in rats over 3 durations of exposure to calculate effective concentrations for pupil constriction [2] and lethality [3]. In the rat study VX was approximately 8-13 times more potent than GB [4] and 11-18 times more potent than GF [5] when miosis was the endpoint. When lethality was the endpoint, VX was approximately 4-25 times more potent than GB [6] and 5-15 times more potent than GF [7]. We have previously identified the effective dosages for miosis [8] and lethality [9, 10] for both GB and GF in the minipig. Our first objective of the current study is to determine the effective dosages for miosis and lethality of VX vapor in minipigs at three exposure durations as a comparison to the current known values for GB and GF.

Constriction of the pupils (miosis) is often identified as the first noticeable sign of exposure to vapor nerve agents because it is caused by a local inhibition of cholinesterase rather than a systemic one and can occur at dosages much lower than those known to cause lethality. For example, in minipigs there is a 30-40 fold difference in vapor dosages of sarin (GB) that elicit miosis vs. those vapor dosages that are potentially lethal [8, 9]. The ratio for miotic vs. lethal vapor dosages ranges from 100-135 fold when cyclo-sarin (GF) is the nerve agent [8, 10]. In rats, dosages that resulted in lethality were at least 90 fold higher than dosages that resulted in pupil constriction [2, 3]. The ratio varied widely depending on exposure duration of exposure. Traditionally, the military and other organizations dealing with inhalation toxicology have accepted Haber's principle of dosage. The relationship, known as Haber's Rule [11], is described by the equation C x T = k, where C is equal to the atmospheric concentration of the chemical being tested, T is equal to the duration of exposure, and k is a constant for some effect or response. This equation assigns equal importance to concentration and time in determining the response. Thus, the product of C x T would remain constant regardless of the concentration or exposure time. This assumption proved to be inadequate for many chemicals, including nerve agents, when attempting to describe cumulative toxicity effects. Thus, the equation was modified to better describe the relationship between concentration and exposure time for a given chemical [12]. The equation C^n x T = k includes the exponent n which is referred to as the toxic load exponent. The toxic load exponent is an experimentally determined, chemical specific value which helps describe the non-linear relationship between concentration and duration of exposure. In practical terms a toxic load exponent that is statistically greater than 1 indicates that concentration has more effect on the biological endpoint. The toxic load exponents for GB and GF vapor exposures in both rats and minipigs have been statistically greater than 1 regardless of the endpoint [4; 5, 6, 7, 8, 9, 10]. This was also the case in rats for VX vapor exposures when pupil constriction was the endpoint [2]. However, when lethality was the endpoint [3] the toxic load exponent was n = 0.92 (with 95% confidence limits of 0.90 to 0.94) suggesting that duration of exposure had more effect on lethality than atmospheric concentration. The second objective of the current study is to determine the toxic load exponents for pupil constriction and lethality for vapor VX exposures in the minipig.

In the current studies sexually mature male Gottingen minipigs were exposed to various concentrations of VX vapor in order to determine the lethal (LCT_{50}s) and effective concentrations (ECT_{50}s) for miosis over exposure durations of 10, 60 and 180 minutes. Male Gottingen minipigs were also exposed to liquid VX via

intravenous (IV) or subcutaneous (SC) injections. Pigs have been found to be similar in anatomy and physiology to humans [13].

2. MATERIALS AND METHODS

2.1. Gottingen Minipigs.

Male and female Gottingen minipigs were obtained from Marshall Farms USA (North Rose, NY). Upon arrival at the testing facility, the minipigs underwent an initial health examination by the attending veterinary staff. The pigs were then quarantined for at least three days. After this time the involved research personnel familiarized the pigs to various procedures that included daily handling, change of location within the animal facilities and adaptation to a sling apparatus. While the animals were in their cages their existence was enriched by human interaction and unfettered access to play toys (hanging chains, bunny balls) or food treats on a daily, rotating schedule.

2.2. Surgical Procedure.

Surgeries to implant cannulas into the external jugular vein were performed on all pigs used in these studies in order to asses VX regeneration from blood samples as well as the inhibition of whole blood acetylcholinesterase (AChE) and butylcholinesterase (BuChE). A complete description of the surgical procedures can be found in previous work [8]. No BuChe, AChE or VX regeneration data will be presented in this manuscript.

2.3. Sling Apparatus.

A sling was used to restrain each minipig during the exposure to the VX nerve agent vapor. The frame of the sling was constructed of airtight stainless steel pipe and Swagelok™ fittings. The slings were custom designed (Lomir Biomedical, Inc., Malone, NY or Canvas and Awning supplies, White Marsh, MD) to fit the build and size of the minipigs. The body of each sling was made of canvas, which contained 2 holes on each side for legs so that it fitted comfortably around the pig. The sling also had two 2 straps that secured over the shoulders and hips. A muzzle harness was placed over the snout and secured both laterally and ventrally to the stainless-steel framing in order to prevent the pig from moving its head from side-to-side. The harness was fitted so that it did not interfere with the pig's ability to breathe.

2.4. VX Vapor Generation

O-ethyl-S-[2-(diisopropylamino) ethyl] methylphosphonothiolate (VX or EA 1701) was used for all vapor exposures. VX was received from the Chemical Transfer Facility at Aberdeen Proving Ground, MD in individually sealed 5- mL ampoules (Lot #VX-U-1243-CTF-N) and certified as chemical agent standard analytical reagent material (CASARM). Seven iterations of a ^{31}P NMR analysis were performed according to an established method [14] to certify the purity of the material as 93.6 ± 0.5 mole percent pure. A high purity grade of triethylphosphate (99.9%; Aldrich Cat. No.: 24,089-3) was used as the internal standard for the VX purity assays. All external standards for VX vapor quantitation were prepared daily with isopropanol (IPA) solvent (Burdick & Jackson Cat. No.: 323-4 purity > 99%).

Saturated VX vapor streams were generated by a continuous flow of nitrogen carrier gas (328-1606 sccm/min) through a multi-pass saturator cell (Glasssblowers Inc., Turnersville, NJ) that contained approximately 5ml of liquid VX. The saturator cell consists of a 100 mm long, 25-mm o.d. cylindrical glass tube with two (inlet, outlet) vertical 7-mm o.d. tubes connected at each end. The main body of the saturator cell contains a porous, hollow ceramic cylinder that serves to increase the contact area between the liquid VX and the nitrogen. The saturator cell allows nitrogen to make three passes along the surface of the wetted ceramic cylinder (Alundum® fused alumina, Norton Co., Colorado Springs, CO) before exiting the outlet arm of the glass cell. The saturator cell body was immersed in a constant temperature bath (Thermo NESLAB, Portsmouth, NH) containing mineral oil so that a combination of nitrogen gas, flow rate and temperature could regulate the amount of VX vapor entering the inhalation chamber. The bath was maintained at 50-107.9°C depending upon the required concentration of VX and the outlet arm of the saturator cell was wrapped in heat tape and maintained at 10°C higher than the mineral bath. The entire apparatus was contained within a generator box that was mounted at the top of the inhalation chamber and maintained under negative pressure. When the chamber was not in use it was necessary to maintain a continuous flow (1 to 2 ml/min) of VX vapor through the chamber in order to preserve the passivity of the chamber. This allowed for generation and maintenance of stable chamber concentrations over a concentration range of 0.001-4.0 mg/m^3.

2.5. Inhalation Chamber.

The minipig whole-body exposures were conducted in a 1000-L dynamic airflow inhalation chamber. The Rochester style chamber was constructed of stainless steel with glass or Plexiglas windows on each of its 6 sides. The interior of the exposure chamber was maintained under negative pressure (0.25-0.30" H_2O), which was monitored with a calibrated magnehelix (Dwyer, Michigan City, IN). A thermoanemometer (Model 8565, Alnor, Skokie, IL) was used to monitor chamber airflow at the outlet.

A quantitative technique using solid sorbent tubes (Tenax/TA) to trap VX, followed by thermal desorption was used to monitor and analyze the VX vapor concentration in the chamber.

The solid sorbent tube sampling system consisted of a 20:35 mesh Tenax-TA fast flow sorbent tube (Dynatherm part number AO-06-2717) and a thermal desorption unit (TDU; ACEM-900, Dynatherm Analytical Instruments, Kelton, PA.) coupled to a gas chromatograph with flame photometric detection (GC/FPD). Samples were drawn from the middle of the exposure chamber, after the chamber attained equilibrium (t_{99}), by inserting a rod containing a sampling tube through small access ports located on the walls of the chamber. The rod was hooked to a vacuum line that drew a sample through the tube at a rate of 3-5 liters/min for 1-9 minutes depending upon the chamber concentration. Sample flow rates were controlled with calibrated mass flow controllers (Matheson Gas Products, Montgomeryville, PA) and verified before and after sampling with a calibrated flowmeter (DryCal, Bios Int'l, Pompton Plains, NJ) connected in-line with the sample stream. The sample tube was transferred to the TDU and prepared for injection onto a Restek RTX-5 column (15m x 0.32mm x 0.5 μm). Temperature and flow programming within the TDU desorbed VX from the sorbent tube directly onto the GC column. Detection was performed with flame photometric detection in the phosphorous mode. Physical parameters (chamber airflow, chamber temperature, and relative humidity) were monitored during exposure and recorded periodically.

The sampling system was calibrated by direct injection of external standards onto the sorbent tubes prior to insertion into the TDU and analysis with GC/FPD. In this way, injected VX standards were put through the same sampling scheme as the chamber samples. A linear regression fit ($r^2 = 0.999$) of the standard data was used to calculate the VX concentration of each chamber sample.

Concentration uniformity was checked at several locations throughout the chamber, including areas directly above the animal.. At higher generated agent concentrations, vacuum pumps were used to draw air through

glass fiber, filter pads at high flow rates to test for the presence of aerosols. Analysis of the glass fiber pads required isopropanol desorption and liquid extract injection onto a 20:35 mesh Tenax-TA fast flow sorbent tube. The sorbent tube was thermally desorbed and analyzed by GC/FPD.

Intravenous and Subcutaneous VX exposures

Prior to beginning the experiments, male pigs were weighed and the dosage of injected VX was given on a per body weight basis. The pigs were injected with liquid VX intravenously, through a 2nd catheter placed in a leg vein, or subcutaneously under the skin behind the ear. Neat VX was diluted in saline to the concentration desired in order to deliver the correct dose of nerve agent. Intravenous injections were delivered as a single bolus in a total volume of 1 ml. The catheter was then flushed with 3 mls of saline to clear the residual VX from the line. Subcutaneous injections were delivered as a single bolus in a total volume of 0.5 ml. We used the up-and-down procedure to choose concentrations for injections. None of the pigs were anesthetized before or during exposure.

2.6. Infrared Pupillometry for assessing pupil constriction

The use of infrared pupillometry to quantitate reduction in pupil size is described thoroughly in previous reports [8]. The basis of infrared pupillometry is the reflection of IR light off the retina and back through the pupil to the camera producing an image of a bright pupil surrounded by a dark iris. The IR method does not cause constriction of the pupil and allows pupil area measurements to be made under dim light conditions. Quantifying pupil area is preferred over a simple measurement of pupil diameter since it is directly proportional to the quantity of light entering the eye, regardless of the shape [15]. A Sony CCD black and white video camera (model XC-ST50), equipped with (2) IR 100-candlepower spotlights was focused on the animal's left pupil for the duration of the nerve agent exposure. The distance between the camera and the animal's eye was standardized at approximately 40 inches and the images were shot externally through Plexiglas windows of the exposure chamber at a constant angle.

Sequential images of the eye, under dim light conditions (<10 candlepower), were digitally captured for analysis and calculation of pupil area using a custom designed software program [8, 16]. VX exposures were for 10, 60 or 180 minutes. However, the pigs were required to remain in the exposure chambers for an additional 15 minutes of out-gassing and for an additional 50-60 minutes to ensure there was no further decrease in pupil area. Miosis was defined as a 50% reduction in pupil area (as compared to baseline) at any time during the VX exposure or during the observation period following exposure.

2.7. Design and Data Analysis.

To determine the progression of experimental exposure concentrations, the up-and-down method with an assumed probit slope of 10 was used [17]. In the miosis studies the binary response used for executing this method was the presence or absence of miosis. In the lethality studies (inhalation, intravenous or subcutaneous), the binary response used for executing this method was dependent on the survival of the minipig for 24 hr after exposure to the nerve agent. The signs of nerve agent exposure were designated as moderate, severe, or lethal. A minipig was classified as having severe signs of exposure if it were gasping, prostrated, collapsed, or convulsing. Muscle tremors, salivation, lacrimation, or miosis constituted a moderate exposure.

For each exposure duration dataset, the method of maximum likelihood estimation (MLE) [18] was used on the resulting quantal data to calculate ECT_{50} values for miosis and LCT_{50} values (and associated asymptotic 95% confidence intervals). Also, MLE was used with a ternary response scale (less than severe, severe and lethal) in order to get estimates for ECT_{50} values for severe effects [9]. Ordinarily, the number of animals used in an experiment utilizing the up and down method is not enough to permit reliable estimation of the probit slope. However, data from several up-down experiments can be combined together to form a subject pool large enough to estimate the probit slope. The resulting dataset can then be analyzed via traditional probit analysis [19] or ordinal logistic regression [5, 20, and 21] in order to obtain a probit slope estimate.

Equations [1] and [2] were used to model the response distribution:

$$Y_N = (Y_P - 5) = k_0 + k_C(\log_{10} C) + k_T(\log_{10} T) \quad (1)$$

$$Y_N = (Y_P - 5) = k_0 + k_C(\log_{10} C) + k_T(Time) \quad (2)$$

where Y_N is a normit; Y_P is a probit; the k's are fitted coefficients; C is vapor concentration; and both T and Time represent exposure-duration. In eq 1, exposure-duration is treated as a covariate (T), whereas in eq 2, exposure-duration is treated as a 3-level factor (Time). The constants, k_C and k_T, are the probit slopes for concentration and Time, respectively. The toxic load exponent, n, is the ratio k_C/k_T. If this ratio is not different (with statistical significance) from 1, then Haber's rule is appropriate for modeling the toxicity. Otherwise, the toxic load model (C^nT) is the proper approach, assuming that there is no significant curvature in the data used to fit the model. Should significant curvature exist, the toxic load model is not appropriate, but it is still superior to Haber's rule in modeling the data.
The present study has exposure durations of 10, 60, and 180 min. For each of the exposure durations, 6 or 7 minipigs were used. Statistical analysis routines, contained within Minitab® versions 13 and 14 (Minitab, Inc., State College PA), and an in-house developed spreadsheet program were used for the analysis of the data.

3. RESULTS

3.1. Animals.

A total of 35 male minipigs were exposed to concentrations of VX vapor to estimate ECT_{50} (miosis), ECT_{50} (severe) and LCT_{50} values. At the time of the surgeries too implant external jugular cannulas, the 35 males, weighed an average of 10.07 kg ± 0.15 (SEM) kg. An additional 14 minipigs were used to establish ED_{50} (severe) and LD_{50} values for intravenous and subcutaneous exposures VX exposures. At the time of the surgeries, the 14 males, weighed an average of 10.77 kg ± 0.26 (SEM) kg. The average weight on the day of the intravenous or subcutaneous VX exposures was 11.95 kg ± 0.21 (SEM) kg. VX injected intravenously or subcutaneously was given on a per body weight basis.

3.2. Median Effective (miosis) and Median Lethal Dosages.

3.2.1. Inhalation

Two different endpoints were used in the analysis of the inhalation effects of vapor exposures to VX, miosis and lethality. The method of maximum likelihood estimation (MLE) was used to calculate ECT_{50} (miosis) and LCT_{50} values (and associated asymptotic 95% confidence intervals) in male pigs exposed to VX vapor for

10, 60, or 180 min. In the studies designed to calculate an ECT_{50} for pupil constriction an animal was classified as having pupil constriction if the pupil area decreased to 50% or less of the baseline value. Table 1 contains EC_{50}, LC_{50}, ECT_{50} and LCT_{50} values for vapor VX exposures for the 2 different endpoints (miosis and lethality) along with 95% Confidence limits. Because of the lack of animals classified as severe at the 10-minute duration of exposure we were unable to calculate ECT_{50} (severe) values for all durations of exposure. However we could calculate a lethal to severe ratio based on the results from the 60 minute and 180 minute duration of exposure.

Table 1. VX MLE for Median Effective Concentrations and Dosages (with approximate 95% confidence intervals on the dosages)

	VX Miosis			VX Lethality		
Exposure (minutes)	**EC_{50}**	**ECT_{50}**	**95% Limits**	**LC_{50}**	**LCT_{50}**	**95% Limits**
10	**0.707**	**7.07**	**6.30-8.18**	**3.41**	**34.1**	**25.7-45.2**
60	**0.144**	**8.65**	**7.80-8.82**	**0.87**	**52.0**	**40.4-66.9**
180	**0.101**	**18.18**	**16.02-19.62**	**0.31**	**55.6**	**41.3-75.1**

Figure 1 display the ECT_{50} and LCT_{50} values for vapor VX and GB exposures (taken from Hulet et al., 2006a & b) plotted vs. duration of exposures. To model the probabilities of miosis or lethality as a function of exposure concentration and exposure-duration several models were fit to the quantal data. Neither the ECT_{50} (miosis) nor LCT_{50} values are constant over time. The toxic load exponent for miosis as an endpoint was n=1.49 (with a 95% confidence interval of 1.07 to 1.91). Because this interval does not overlap one, we have proved that pig miosis does not obey Haber's rule. The toxic load exponent for lethality as an endpoint was n=1.13 (with a 95% confidence interval of 1.03 to 1.23). Because this interval does not overlap one, we have proved that pig lethality does not obey Haber's rule. Potential curvature in both data sets were evaluated by inserting a $(\log T)^2$ term into the model. These terms were found to be statistically insignificant. For executing the up and down method in these studies, the probit slopes on concentration, kC, were assumed to be 10 (see Section 2.9). The probit slope of the best model fit for miosis as an endpoint was 10.1 ± 9.6. The probit slope of the best model fit for the lethality as an endpoint was 17.7 ± 10.9. The confidence intervals of the probit slopes for both endpoints are not statistically different from the assumed value of 10.

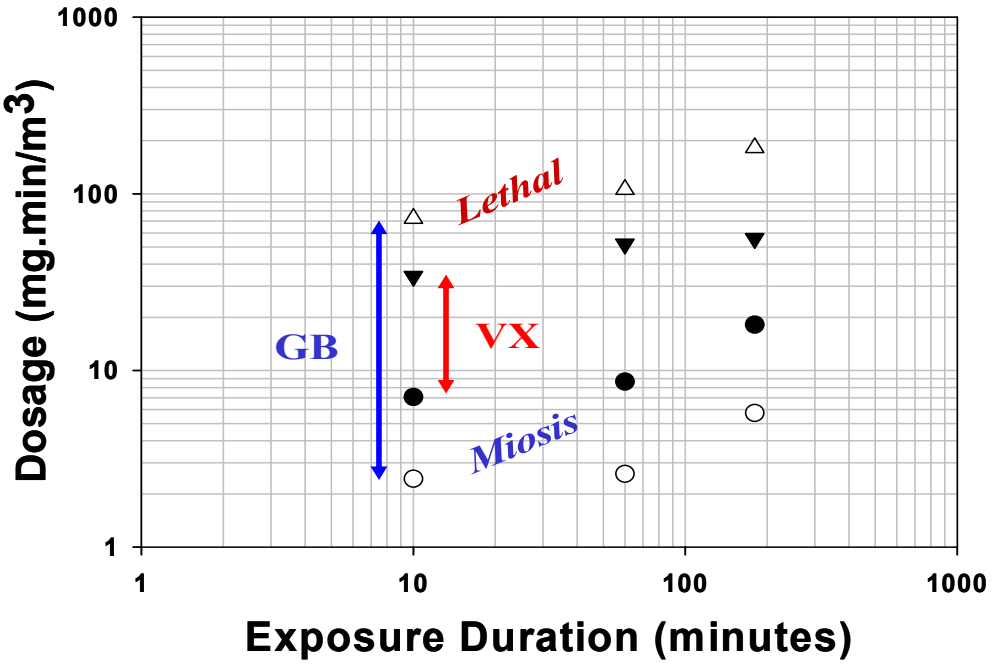

Figure 1. MLE ECT_{50} and LCT_{50} Estimates as a Function of Exposure Time. Effective dosages for miosis are depicted for VX (●) and GB (○) in comparison to the effective lethal dosages for VX (▼) and GB (Δ). GB values were taken from Hulet et al., 2006 [9, 10].

GB was 2.9, 3.3 and 3.16 times more potent than VX at causing miosis for the 10, 60 and 180 minute exposures. In contrast VX was 2.47, 2.29 and 3.37 more potent at causing lethality than GB for the same durations of exposures. The ratio of ECT_{50} (miosis) values to LCT_{50} values for the 2 nerve agents are shown in Table 2. In minipigs VX vapor is less potent than GB at causing pupil constriction. Additionally, the onset of pupil constriction is delayed in comparison to onset upon exposure to GB. VX vapor was more potent than GB when lethality was the endpoint. However, unlike with GB vapor exposures, constriction of the pupils was not the definitive first noticeable effect upon a potentially lethal VX vapor exposure. The ratio of LCT_{50} to ECT_{50} (miosis) values for vapor VX exposures ranges from approximately 3-5.5 fold while the same ratio for GB exposures ranges from approximately 30-40 fold. The ratio of LCT_{50} to ECT_{50} (severe) for vapor VX exposures was 1.12.

Table 2. Ratio of LCT_{50} to ECT_{50} (miosis) values for GB and VX vapor exposures

Exposure Duration (min)	LCT_{50}/ECT_{50} (miosis) ratio	
	GB	VX
10	29.7	4.2
60	40.6	5.4
180	31.7	3.4

3.2.2. Intravenous and Subcutaneous VX exposures

Male Gottingen minipigs were also exposed to liquid VX via IV or SC injections. Signs of nerve agent exposure were classified as lethal, severe, or moderate. Maximum likelihood estimation using a probit model was used to calculate the median effective (severe effects) and lethal doses (LD_{50}) for IV and SC exposures. For intravenously injected VX, the LD_{50} was 11.8 µg/kg (9.7 – 14.5 µg/kg) and the ED_{50} (severe) was 6.6µg/kg (4.8 – 9.0 µg/kg). For subcutaneously injected VX, the LD_{50} was 16.1µg/kg (12.9 – 19.9 µg/kg) and the ED_{50} (severe) was 12.8µg/kg (10.1 – 16.2 µg/kg). Table 3 summarizes these results with a comparison to GB [9]

Table 3. MLE ED_{50} (Severe) and LD_{50} Estimates for VX intravenous and subcutaneous exposures.

	Route	LD50 (µg/kg)	95 % limits (µg/kg)	ED50 (severe) (µg/kg)	95 % limits (µg/kg)
VX	intravenous	11.8	9.7-14.5	6.6	4.8-9.0
	subcutaneous	16.1	12.9-19.9	12.8	10.0-16.2
GB	intravenous	16.1	11.2 - 23.1	10.0	7.5 – 13.5
	subcutaneous	36.8	26.2 – 51.6	18.9	8.8 – 40.6

4. DISCUSSION

Most research on VX focuses on percutaneous hazards, resulting in a limited amount of available data on the hazards of inhaled VX as a vapor. LCT_{50} values for whole body exposures using VX as an **aerosol** have been established for mice, rats, hamsters, guinea pigs, rabbits and dogs [22, 23]. Whole body VX **vapor** LCT_{50} values of 4.0 mg.min/m^3 [24] and 16.1 mg.min/m^3 [25] have been established in mice. Benton et al. [3] determined an LCT_{50} value of 48.5 mg.min/m^3 in rats exposed to VX as a vapor for 10 min. The LCT_{50} for a 10-minute VX vapor exposure in the current study was 34.1 mg.min/m^3, with a confidence interval that overlaps that of Benton's rat values. In contrast the LCT_{50} for a 10-minute GB vapor exposure [9] in minipigs (72.5 mg.min/m^3) was approximately 3 times lower than the LCT_{50} value (231 mg.min/m^3) in rats [26]. The differences between LCT_{50} values seen between pigs and rats when exposed to GB but not to VX are most likely a reflection of the much greater circulating carboxylesterase (CaE) levels in rats than in pigs and that VX is 500-fold less reactive with CaE than are the other nerve agents [27].

In rats, [3] vapor VX is approximately 4-25 times more potent than vapor GB [26] and 5-15 times more potent than vapor GF [7] when lethality is the endpoint. In the current study the LCT_{50}s for vapor VX exposures were 34.1, 52.0 and 56 mg.min/m^3 for 10, 60 and 180 minutes, respectively. Vapor VX was therefore approximately 2-3 times more potent as a lethal agent than GB [9] in minipigs. In humans VX is estimated to be approximately twice as toxic as GB by the inhalation route [28].

Constriction of the pupils (miosis) is often identified as the first noticeable sign of exposure to vapor nerve agents. Callaway and Dirnhuber [29] investigated the miotic potency of VX in rabbits. They were able to

estimate an ECT_{50} of 0.04 mg.min/m^3. This value was significantly more potent than the ECT_{50} they determined for GB (1.32 mg.min/m^3). In the same species, Benton et al. [2] found that VX was approximately 8-13 times more potent at causing pupil constriction than GB [4] and 11- 18 more potent than GF [5]. Surprisingly, in minipigs VX vapor is less potent than either GB or GF [8] at causing pupil constriction. Additionally, in the current study the onset of pupil constriction was delayed for VX exposure in comparison to the onset upon exposure to GB. Others [29, 30] have noted a similar delayed onset of pupil constriction upon vapor exposure to VX than is seen for GB.

We have previously identified that in minipigs there is a 30-40 fold difference in vapor dosages of sarin (GB) that elicit miosis vs. those vapor dosages that are potentially lethal [8, 9]. The ratio for miotic vs. lethal vapor dosages ranges from 100-135 fold when cyclo-sarin (GF) is the nerve agent [8, 10]. The ratio of ECT_{50} (miosis) vs. LCT_{50} values for vapor VX exposures ranges from 3-6 fold. Therefore, in the minipig the dosages of VX vapor that elicit pupil constriction are much closer to the dosages that result in severe toxic signs or lethality than are seen for either GB or GF vapor exposures. Additionally, constriction of the pupils was not always the definitive first noticeable effect upon a potentially lethal VX vapor exposure, as is the case in GB or GF exposures.

In the current studies the toxic load exponents for miosis and lethality were both significantly greater than 1 indicating that the concentration of the exposures had more of an impact on the endpoint than did the duration of exposure. This was also the case in rats for VX vapor exposures when pupil constriction was the endpoint [2]. However, when lethality was the endpoint [3] the toxic load exponent was n = 0.92 (with 95% confidence limits of 0.90 to 0.94) indicating that duration of exposure had more effect on lethality than atmospheric concentration. A likely explanation for these findings is suggested by the authors who hypothesize that delayed percutaneous absorption or oral ingestion is causing the greater effects of duration of exposures in comparison to concentration. While our study did nothing to counteract the possible effects of percutaneous absorption, we can effectively rule out oral ingestion. The lack of oral ingestion by pigs may explain the differences in toxic load values between the pig and rat when lethality is the endpoint.

The historical LD_{50} for intravenous VX exposures in pigs is 9 ug/kg [31]. In the current study for intravenously injected VX, the LD_{50} was 11.8 µg/kg (9.7 – 14.5 µg/kg) and the ED_{50} (severe) was 6.6µg/kg (4.8 – 9.0 µg/kg). For subcutaneously injected VX, the LD_{50} was 16.1µg/kg (12.9 – 19.9 µg/kg) and the ED_{50} (severe) was 12.8µg/kg (10.1 – 16.2 µg/kg). Not surprisingly, VX was more potent than either GB or GF (Hulet et al., 2006b &c) by both routes of exposure.

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