THE INHALATION TOXICITY OF VX AEROSOLS ASSESSED IN THE MCNAMARA GLOVE BOX FACILITY

John C. Carpin, David A. McCaskey and Kenneth P. Cameron

U.S. Army Edgewood Chemical Biological Center
AMSRD-ECB-RT-TT
5183 Blackhawk Rd.
Aberdeen Proving Ground, MD 21010-5424

A series of mouse nose-only inhalation exposures with VX were conducted in the recently established McNamara glove box facility for the purpose of providing LCt_{50} reference data for future studies in this facility and to serve as a benchmark for ranking the toxicity of other agents. Neat VX challenge aerosols were generated by feeding micro-liter quantities of agent from a loaded syringe to a custom-made air assist atomizer. Exposure concentrations were assessed by both real-time aerosol monitor and gas chromatograph analysis of chamber filter pack samples. Exposures were run for 10 minutes in duration with 4 animals per test. An "up and down" paradigm was used to sequentially select target agent concentration levels from one exposure run to the next based on the resulting 24 hour lethality fraction. Chamber sampling results indicated that over the concentration range tested, the agent existed primarily in the aerosol phase most likely because of the brief transit time between the atomizer and the chamber exposure ports which did not allow sufficient time for vapor equilibration. The resulting LCt_{50} for neat VX aerosol in the mouse via the inhalation route was significantly lower than anticipated based on another recent nose-only study in which mice were challenged with saline aerosols laced with VX. Overall our results indicate that the LCt_{50} for VX aerosol in the mouse via the nose-only exposure method is comparable to previously reported values for head only and whole body exposures under conditions of vapor and aerosol challenge.

INTRODUCTION

The McNamara Glove Box Facility (MGBF) was developed out of Tech Base for the purpose of assessing the inhalation toxicity of emerging chemical threat materials. The facility houses a 12 port rodent nose-only exposure chamber. The nose-only (i.e. inhalation only) exposure approach offers some advantages for agent screening from an operational standpoint. On one hand, it requires a much smaller quantity of agent per exposure test than traditional whole body exposure methods thereby reducing cost and improving safety. In addition, it is inherently safer for those involved with post exposure animal handling by minimizing external body contamination of the test subjects. Despite these advantages, the nose-only (i.e. inhalation only) method of exposure has been rarely used for nerve agent inhalation

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
01 OCT 2005	**N/A**	**-**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
The Inhalation Toxicity Of Vx Aerosols Assessed In The Mcnamara Glove Box Facility	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **U.S. Army Edgewood Chemical Biological Center AMSRD-ECB-RT-TT 5183 Blackhawk Rd. Aberdeen Proving Ground, MD 21010-5424**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES
See also ADM001851, Proceedings of the 2003 Joint Service Scientific Conference on Chemical & Biological Defense Research, 17-20 November 2003., The original document contains color images.

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**UU**	**10**	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std Z39-18

screening in the past most likely because it excludes the percutaneous and ocular routes of agent exposure. Consequently, a series of inhalation tests were planned with the classical agent VX in rodents using the nose-only exposure method in order to establish a reference point for ranking the toxicity of other agents in the MGBF. The data presented here represent an initial installment of VX inhalation toxicity data in the mouse model.

METHODOLOGY

MATERIALS

Nerve agent VX (O-ethyl S-[2-diisopropylaminoethyl] methylphosphonothiolate), at a purity of 96.3 %, was used for all exposure tests in this study. In addition, distilled GF (cyclohexyl methylphosphono-fluoridate), at a purity of 99.9%, was used as an internal standard in all analytical work performed. Isopropyl alcohol (IPA) was the general solvent used for all standard preparation, filter/substrate extraction and bubbler collection procedures. The IPA was rated to be greater than 99.5% pure.

EXPERIMENTAL DESIGN

Exposure tests on each species were conducted in two phases: a range finding (RF) phase, and a second series (SS) phase. The purpose of the range finding phase was to locate the 0 to 100% lethal response window. This was accomplished by exposing groups of 4 animals for 10 minutes to a series of agent concentrations selected on the basis of a modified “up and down” approach. The initial test concentration was chosen near the estimated 50% lethality point of the test compound based on available data. Subsequent target exposure concentrations were incremented up or down in relation to the previous test concentration depending on the 24-hour (overnight) post exposure response that resulted from the latter. A maximum of 20 animals for any one species were used for the range finding phase. A "second series" of exposure tests were conducted using a second batch of test animals to fill in data points in the 0 to 100% response window, to tighten the confidence limits, and to compensate for any exposure level targeting problems that occurred during the range finding phase. A maximum of 24 animals was used for the follow-up phase with four animals per exposure test.

EXPOSURE CHAMBER

Animal exposures were conducted using a dynamic exposure system (Figure 1) housed in the primary compartment of the MGBF. The central component of this system is a 12-port rodent nose-only exposure chamber. The exposure chamber is of stainless steel construction and consists of two compartments, a low volume (<100 cm^3) inner plenum enclosed by an outer cylindrical shell. Airborne agent enters the plenum through a single inlet at the bottom of the chamber and flows through 12 delivery "trumpets" spaced equally around its circumference. The trumpets uniformly distribute the flow of agent towards the center of matching exposure ports built into the outer cylindrical shell. The chamber exposure ports are designed to accept an animal restraint tube or sample device adaptor. Agent exits the chamber from a single outlet port located at the top of the outer compartment.

Restraint tubes are used to immobilize an animal and position the animal’s nose within an exposure port. Each mouse restraint tube consists of an open ended transparent lexan® body with a stainless steel nose cone at the opposing end. A plastic locking cap with rubber bushing and adjustable stainless steel plunger is placed on the open end of the tube to hold the mouse is in place. Animals are placed in the tubes approximately 15 minutes prior to exposure and removed immediately after exposure.

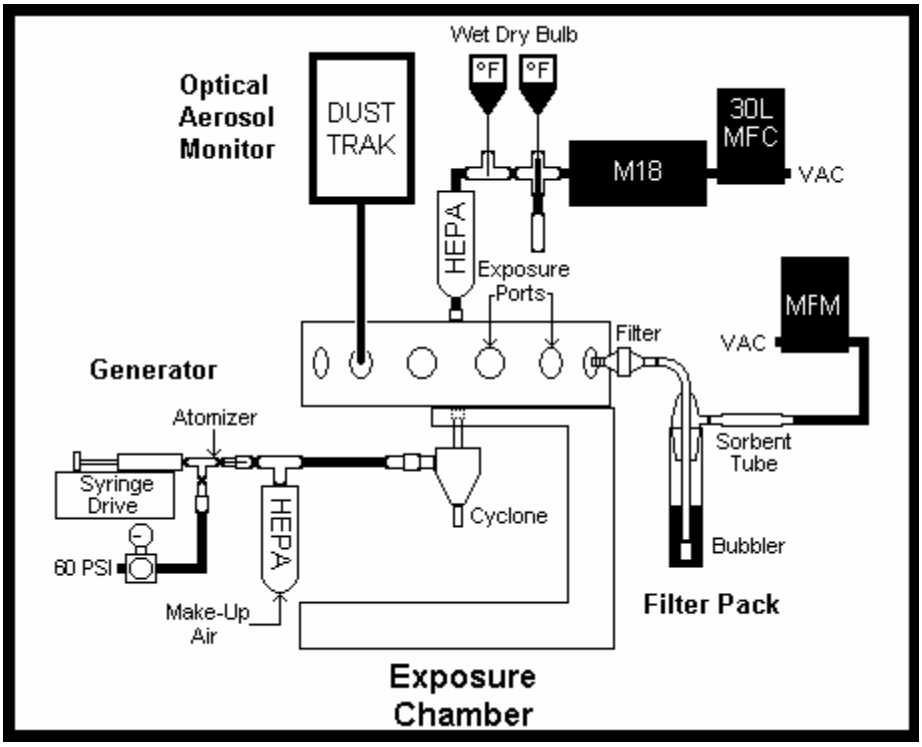


Figure 1. Schematic of Exposure System

AEROSOL GENERATION

VX challenges were generated by feeding micro-liter quantities (10 to 50 ul) of neat agent with a syringe drive (Model 22, Harvard Apparatus Inc., South Natick, MA) to a custom designed airblast atomizer. The atomizer itself was of a dual needle design with a liquid agent feed needle mounted coaxially inside a larger outer needle to which compressed air was delivered at 60 psig. The resulting aerosol flow (~2 lpm) was mixed with filtered make-up air to achieve a total flow of 24 lpm through the exposure chamber. Prior to entering the exposure chamber, the agent stream passed through a cyclone separator designed to remove particles greater than 2 μm.

MONITORING OF EXPOSURE ATMOSPHERES

Three methods were employed to characterize exposure chamber atmospheres: 1) the collection of agent directly onto filter packs followed by subsequent analysis of the samples for agent content; 2)the monitoring of chamber aerosol concentration in real time using an optical aerosol mass monitor; 3) the analysis of aerosol particle size distribution by means of cascade impactor.

FILTER PACKS

Measurement of average agent concentration in the exposure chamber was accomplished by filter pack sampling directly from two opposing exposure ports, simultaneously. Each filter pack was designed to sample both aerosol and vapor phase agent and consisted of three collection elements in series: a high efficiency particulate filter (Gelman type A/E glass fiber 25 mm) mounted in a stainless steel holder, a glass midget bubbler containing 15 mls of IPA, and a sorbent tube (glass) packed with Tenax TA. All filter pack sample flows were generated by means of a vacuum pump located in the glove box and regulated with individual mass flow controllers (Matheson model 8272) at 1.0 or 1.7 liters per minute. Sample flows were set immediately prior to an exposure by measuring them with an electronic bubbler meter (Sensidyne Gillibrator 2). Sample flow calibrations were done with bubblers primed with IPA and sorbent tubes in-line.

OPTICAL AEROSOL MONITOR

The DUSTRAK™ aerosol monitor (TSI, Inc., St. Paul, MN), a portable laser photometer that uses light scattering technology to measure airborne particle concentrations in real time, was used to sample agent aerosol directly from a chamber exposure port. The instrument samples air under the power of an internal pump at a flow preset 1.7 liters per minute and is capable of measurement over a wide range of aerosol concentrations (100 mg to 10 ug/m3). The resulting aerosol mass concentration displayed by the instrument is equal to the product of the voltage signal and an internal calibration index. Mass concentration data is updated at one second time intervals and is also stored in memory for later downloading.

To use the DUSTRAK™ for quantitative agent aerosol mass concentration measurements, the appropriate calibration index must be determined for the particular aerosol being measured. This is done by relating the instrument mass concentration reading to the actual mass concentration as measured by an independent reference method using the following proportionality

$$\text{New Calibration Index} = \frac{\text{Reference average concentration}}{\text{DUSTRAK average concentration}} \times \text{Preset Calibration Index} \qquad (1)$$

For the purpose of this study, the Reference concentration is the average agent mass concentration as measured by GC-FPD analysis of the particulate filter component of the filter pack samples. Using equation 1, a calibration index was determined for each exposure run and these values were later averaged to determine an overall calibration index for the VX aerosols.

AEROSOL PARTICLE SIZING

Aerosol particle size distribution was assessed with a SS seven stage cascade impactor (In-Tox Products, Inc., Moriarty, NM) using SS substrates lightly coated with vacuum grease. The selective size ranges for this device at the operating flow of 1 lpm were as follows: > 4.6 um, 3-4.6 um, 2.09-3.0 um, 1.62-2.09 um, 1.06-1.62 um, 0.72-1.06 um, 0.33-0.72 um, < 0.33 um.

SAMPLE HANDLING AND ANALYSIS

Filter pack, impactor and DUSTRAK™ samples were pulled continuously throughout the wash-in, steady state, and wash-out periods of each exposure run. Upon completion, samplers were unplugged from the exposure chamber and the collection substrates were removed and immediately placed in 1 to 4 mls of IPA for agent extraction. All bubblers were topped off to 15 mls with IPA to compensate for evaporative losses during sampling and the solution was transferred to glass vials. A 10 ml portion of each bubbler sample was concentrated to 3 mls by evaporation under N_2 purge. Both the original sample solution and concentrate were later analyzed for agent content.

Quantification of agent mass present in sample solution/extracts was accomplished with a gas chromatograph system (HP Model 6890) equipped with a flame photometric detector operated in phosphorus mode (GC-FPD). VX calibration standards were prepared in IPA over the range of 20.73 to 0.26 ug/liter. All calibration standards and samples were spiked with GF at the 10 ug/ml level to improve the precision of the analysis. The area ratio of the analyte (VX) peak to that of the internal standard (GF) was used to construct calibration curves and subsequently used to determine the VX concentration in an unknown sample.

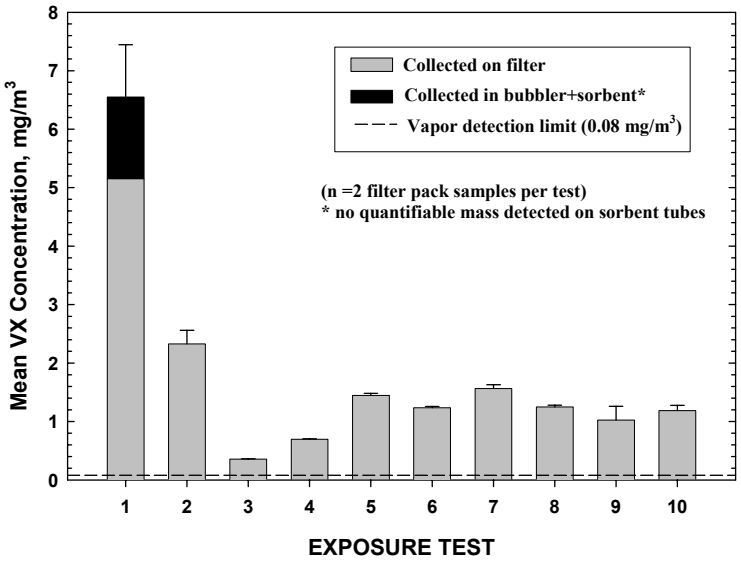
Any agent trapped on the solid sorbent tube component of the filter pack was quantified using a thermal desorption unit (Dynatherm ACEM900) coupled to the GC-FPD system. To calibrate this system, know amounts of external standard (VX/IPA) and internal standard (GF/IPA) were loaded onto a set of blank solid sorbent "calibration" tubes. This was accomplished by injection of the standard directly onto the glass frit of the sorbent tube while purging with ambient temperature helium gas. Samples tubes from the exposure runs were also spiked with internal standard by direct injection as described. The ratio of area counts of VX and internal standard GF obtained from the analysis of the "calibration" tubes were used to construct a calibration curve. This calibration curve was in turn used to determine the quantity of agent on the GF spiked solid sorbent tube chamber samples.

RESULTS

FILTER PACK ANALYTICAL RESULTS

GC-FPD analysis of the filter pack samples provides a quantitative measure of the average agent mass concentration present in the exposure chamber over the duration of an exposure test. In addition, the distribution of agent mass on the individual filter pack components was used to assess the relative proportion of agent present in the aerosol versus the vapor phase. A summary of the results of the filter pack analyses for ten exposure test runs are shown in Figure 2. Mean VX concentration is depicted in terms of two mass components: 1)agent mass collected on the particulate filter element of the filter pack, and 2) agent mass collected in the vapor trap components (i.e. bubbler plus solid sorbent tube) of the filter pack. With the exception of exposure test #1, all quantifiable agent was collected on the particulate filter element of the filter pack. In exposure test #1, which was the highest exposure test concentration, 20 % of the VX was collected in the bubbler component of the filter pack. These results imply that the VX challenge was principally in aerosol form for exposure tests 2 through 10, excluding any vapor which may be present below the bubbler detection limit of 0.08 mg/m^3. A mixed aerosol/vapor phase was present during exposure test #1.

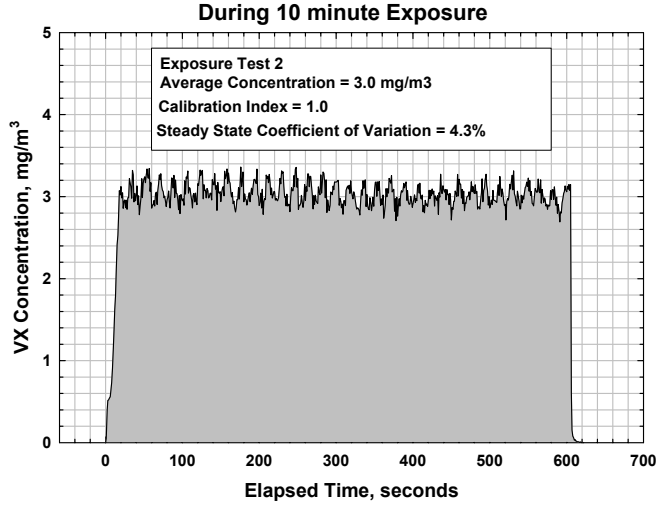
FIGURE 2. Summary of Analytical Results of Filter Pack Samples



OPTICAL MONITORING RESULTS

The DUSTRAK™ aerosol mass monitor was used to sample the agent during an exposure and to indicate the concentration present in the exposure chamber in real time. At the conclusion of an exposure test, a mapping of the concentration-time profile was downloaded from the unit. The data in Figure 3 show a typical aerosol concentration-time profile as represented by exposure test #2. The data show that the exposure concentration profile closely resembles a step function with a characteristic rapid rise and fall. The average time required to reach target steady state concentration for all exposure tests was 15 seconds with a maximum rise time of 22 seconds during any one run. Once the target concentration was reached, steady state concentration could generally be maintained within ± 10% of the target concentration, with a coefficient of variation about the mean ranging from 4.3% to 8.3%.

Figure 3. Chamber Aerosol Concentration Profile During 10 minute Exposure



A DUSTRAK™ optical calibration index was calculated for each exposure test as previously described using Equation (1) in conjunction with the time integrated average VX aerosol concentrations determined by optical monitoring and filter pack sampling. The resulting optical calibration indexes, shown in Table 1, are reasonably consistent with the exception of the index for exposure test #1 which is

TABLE 1. DUSTRAK™ Optical Calibration Index for VX Aerosols

Exposure Test	1	2	3	4	5	6	7	8	9	10	Average* Index
Measured Index	0.38*	0.78	0.70	0.70	0.75	0.82	0.77	0.71	0.63	0.64	0.72±0.06

* Exposure test #1 index excluded from average

about a factor of two lower than the other values. Also, it can be shown that the index for exposure test #1 is a statistical outlier. Considering this, along with the fact that during exposure test #1 a significant proportion of agent was present in the vapor state, it was deemed appropriate to exclude this data point in the computation of the overall average calibration index for the VX test aerosols.

AEROSOL SIZE CHARACTERISTICS

Cascade impactor samples were analyzed in accordance with the methods described and the data fit via probit analysis assuming a log-normal distribution function. The results of these analyses are summarized in Table 2. Unfortunately, the inclusion of data from only two impactor runs is possible

TABLE 2. Summary of Aerosol Size Measurements

Exposure Test	AMMD, μm	σg
9	1.51	2.25
10	1.65	2.21

AMMD = Aerodynamic Mass Median Diameter
σg = geometric standard deviation

due to the fact that samples from exposure tests 1 thru 8 showed clear evidence of particle bounce artifact. Coating the impactor substrates with a thin layer of vacuum grease in exposure tests 9 and 10 remedied this problem.

EXPOSURE RESULTS

Tables 3 shows the outcomes in terms of lethality versus exposure concentration of VX for the mouse in order of decreasing toxicity. Data sets from both the range finding and second exposure series are included together. The left hand column lists the exposure concentration-time product (CT) as measured by GC-FPD analysis of filter pack samples. Also, time weighted average concentrations derived from the DUSTRAK™ aerosol monitor data, scaled with average optical calibration index of 0.72, are shown in column two. The lethal fraction of exposed animals are shown for time periods of 24 hours and 14 days post exposure. Generally, any deaths that occurred took place within the first 24 hours post exposure and in many cases within 60 minutes post exposure. There was one delayed death during exposure test #5. During exposure test #1, which had the highest agent test concentration, all test subjects were dead well before the end of the 10 minute exposure period.

TABLE 3. Summary of VX Aerosol Induced Lethality in the Mouse
Exposure Duration = 10 minutes
(RF = range finding; SS = second series)

Concentration, mg/m^3		Lethal Fraction of Exposed		Exposure Test	Exposure Series
Measured by Filter Pack	Measured by DUSTRAK*	Outcome < 24 hrs	Outcome < 14 days		
6.55^	NA	4/4[a]	4/4[a]	1	RF
2.33	2.25	4/4	4/4	2	RF
1.56	1.53	4/4	4/4	7	SS
1.45	1.46	2/4	3/4[b]	5	RF
1.18	1.40	3/4	3/4	10	SS
1.25	1.32	2/4	2/4	8	SS
1.03	1.22	1/4	1/4	9	SS
1.24	1.12	0/4	0/4	6	SS
0.7	0.75	0/4	0/4	4	RF
0.35	0.38	0/4	0/4	3	RF

^ 20% of mass in vapor phase; a - test subjects died ~ 5 minutes into exposure; b - Outcome < 48 hrs; * Based on an average calibration index of 0.72

A summary of the resulting LCT50s, 95% fiducial intervals and dose response slopes as determined by probit analysis are shown in Table 4. Values of these parameters are shown for the pooled data using both the analytically derived and optically derived exposure data. Note that exposure test #1 data was not included in the statistical analysis.

TABLE 4. Statistical Summary of 14 Day VX Inhalation Toxicity of Combined Mouse Exposure Groups

Filter Pack Data			DUSTRAK™ Data		
LCT_{50}^ mg min/m^3 (T=10 min)	95% F.I.	Slope	LCT_{50}^ mg min/m^3 (T=10 min)	95% F.I.	Slope
16.1	12.8 - 19.3	8.7	17.6	16.1 -18.8	25.6

^ Excludes exposure test #1 F.I. = Fiducial Intervals

DISCUSSION

Considering the fact that the volatility of VX is near 10 mg/m3, it is surprising that the VX challenge in this study was principally in the aerosol and not the vapor phase over the range of exposure concentrations. One plausible explanation for this result is that there is likely insufficient time for vapor equilibration to take place in the time it takes for aerosol to travel from the generation point to the exposure port (~ 100 msec). On the other hand, at higher test concentrations (i.e. exposure test #1), the resulting increase in aerosol surface area enhances the evaporation process resulting the presence of more vapor.

The mouse LCT_{50} values determined on the basis of optical monitor and filter pack analysis (Table 4) are equivalent. The optical monitoring data is "tighter', so to speak, in comparison as evidenced by the narrower 95% fiducial indices and steeper slope value. In addition, two reversals that are evident in the 14 day lethal outcomes (Table 3) for the filter pack data are not present in the DUSTRAK results. Thus the optical method appears to better track the biological responses.

The LCt50 determined for VX in the mouse by the nose-only exposure route in this study (17 mg/m3) is considerably lower than previously reported values of 71 mg min/m^3 by $Bide_1$ and 72 mg min/m^3 by $Carroll_2$. This fourfold difference is likely due to differences in test methodologies. Bide disseminated VX in dilute form from a saline solution in contrast to the neat VX dissemination technique used in this study. In addition, the VX concentration was assessed by tracer technique in the Bide study and not by direct quantification of agent. Carroll on the other hand used a static exposure method in which mice were immersed in a chamber containing saturated VX vapor. To achieve nose-only exposure, their bodies were place in a shield tube with the animals nose exposed and the entire tube placed in the chamber. Our results are more in line with the head-only LCT50 of 14 mg min/m3 previously obtained at $Edgewood_3$.

CONCLUSIONS

•The LCt50 obtained for nose-only (i.e. inhalation only) exposure to VX in the mouse appears to be significantly lower than previously reported

•The use of optical aerosol monitoring as a supplemental measurement technique to standard agent sampling methods can be a useful tool in reducing variability in agent aerosol inhalation exposure results

REFERENCES

1. Bide, R. W., Risk, D.J. "Inhalation Toxicity of Aerosolized VX" DRES TR 2000-063 (U), September 2000 (U)

2. Carroll, N.C., F.C.G. Hoskin, M.K. McPhail, D.K. Myers. "Vapour Toxicity of VE and VX to Female Mice." STP 121, 10 May 1957. (U)

3. Koon, William S, J.W. Crook, and F.W. Oberst. CWL Technical Memorandum 24-37, April 1960 (U) April 1960 (U)