THE POTENTIAL NEUROTOXIC EFFECTS OF LOW-DOSE SARIN EXPOSURE IN A GUINEA PIG MODEL

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ABSTRACT

This study is assessing the effects in guinea pigs of repeated low-dose exposure to the nerve agent sarin. Preliminary results suggest no effects of either repeated 0.2 or 0.4 X LD50 sarin exposure (compared with saline) on body weight or temperature, general physical signs, flinch threshold or activity level, or on EEG activity. In contrast, RBC cholinesterase levels dropped to 20% of baseline following the tenth exposure in the 0.4 group. Since this study is ongoing, data from receptor binding and brain cholinesterase assays and histopathology are still being collected and analyzed, and may be influenced by the dramatic changes in cholinesterse activity.

INTRODUCTION

A great deal of research has been conducted to study the single acute effects of chemical warfare nerve agents (CWNA) and how to protect against the acute toxic effects. Consequently, the sequence of events following a single, seizure-eliciting exposure to organophosphorus (OP) CWNA has been relatively well characterized. However, much less is known about the effects of repeated low dose exposure to OP nerve agents, and there has been concern that such exposure may have contributed to the adverse health effects reported by Gulf War veterans. Due to the uncertainty of the effects of prolonged low dose chemical exposure, there is a need to better understand the potential adverse health consequences of such exposure and to determine what level of exposure may produce adverse effects.

The experiments detailed here are intended to furnish initial data that should address a number of these issues and form the basis for further research. These ongoing studies concurrently examine electrophysiological, behavioral, biochemical, neurochemical, and histopathological parameters in an animal model that utilizes repeated exposure to low levels of the nerve agent sarin.

There have been numerous studies of the neurobehavioral effects of repeated low-level exposure to a variety of OP agents such as DFP or paraoxon (see Russell and Overstreet, [15] 1987 for a review), and some limited studies with nerve agents. Previous studies of repeated administration of the nerve agent soman shows that such treatment produces transient, but reversible, changes in regional brain cholin-

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esterase (ChE) activity and regional brain muscarinic receptor (mAChR) numbers.[3, 9] During the time of repeated exposure the animals can display altered behavior and may develop a tolerance to the agent as the exposures continue.[12, 14] In addition, it has been reported that repeated low level exposures to sarin may produce permanent alterations in brain electroencephalographic (EEG) spectrum that far outlast the period of exposure.[2] Because of this persistent change in EEG, there have been continuing concerns that exposure to low doses of nerve agents can also produce neural lesions in brain such as those seen after exposure to high doses of nerve agent that produce prolonged seizures.[7, 8]

In the present study, guinea pigs, previously instrumented to record EEG activity, were exposed daily (5 days/wk for two weeks) to two doses (0.2 X LD50 and 0.4 X LD50) of sarin. Measures of red blood cell (RBC) ChE, EEG activity, body weight, body temperature, flinch thresholds (nociception), and general activity levels were determined during the exposure phase as well as 2 hrs, 3, 10, 30, or 100 days following exposure in different groups.

The two-week period of nerve agent exposure was selected because this period of exposure provides sufficient time for ChE to be driven to a low, stable level. The nerve agent, sarin, and the doses (0.2 and 0.4 X LD50) were selected to allow replication and expansion on a database of low-dose work begun at USAMRICD.[1] These doses, particularly the higher 0.4 dose, also produce no notable adverse physiological effects such as weight loss or lethality. The current LD50 has its basis in protection studies historically conducted in guinea pigs at USAMRICD.

MATERIALS AND METHODS

General Methods: Guinea pigs (Final N = 180) are anesthetized with isoflurane and stereotaxically implanted with stainless steel cortical screw electrodes.[10, 11] Following a one-week recovery period and initial handling days (M, Tu), baseline EEG (30 min/day), baseline blood ChE, and behavioral data is being gathered on two pre-exposure days (W, Th). Animals are then injected daily, s.c., 5 days/wk for 2 wks with saline or sarin (0.2, or 0.4 x LD50; LD50 = 42 ìg/kg). On each day of exposure, body temperature (pre- and post-exposure) and body weight are measured, and the animals are monitored for EEG activity (power spectral analysis, broken into five EEG bands) for 15 minutes to establish a daily pre-exposure baseline. After each injection the animals are monitored for EEG activity for 1 hour and are assessed for general signs of sarin exposure, including eyelid closure, facial tremor, fasciculation, writhing, vocalization, circling, biting, the ease of handling, lacrimation and salivation. Following EEG recording, animals are assessed for change on measures of nociception (flinch threshold/foot shock) and general activity. Blood is drawn on selected days for analysis of RBC ChE activity. After the termination of the exposure phase, separate groups of animals are evaluated for EEG and behavioral changes at 3, 10, 30 and 100 days. In addition, at each of these times (plus at 2 hrs post exposure), groups of animals are euthanized (75 mg/kg, i.p., pentobarbital) and transcardially perfused. The brain and heart are removed, and regional brain ChE activity, regional brain receptor B_{max} and K_d, and brain neuropathology are being determined. [^{3}H]-Pirenzepine is being used for mAChR binding to M_1 receptors,[5] and [^{3}H]-CGP-39653 is being used for glutamatergic NMDA (N-methyl D-aspartate) receptor binding.[16] Separate brain and heart evaluations for histopathological assessment are carried out with a number of staining techniques, including hematoxylin and eosin (H & E) and glial fibrillary acidic protein (GFAP).[6] Apo-tag will be used to identify potentially apoptotic neurons.[13]

Nociceptive (flinch) thresholds are determined by the up-and-down procedure.[4] The animal is placed in a test chamber (16 cm L; 11 cm W; 13 cm H) with a stainless steel grid floor through which varying intensities of electric shock can be delivered. After a 1-min habituation period, single shock pulses (0.5 sec) are delivered at 15-sec intervals. Shock intensities are available from 0.05 to 4.0 mA in 20 steps arranged logarithmically. Flinch is being defined as any visual withdrawal reaction in response

to shock presentation, and shock intensity will be varied according to each response. An adaptation of the "up-down" method for small samples is used for determining the order of presentation of shock intensities during each series. The midpoint of pre-exposure baseline measurements serves as the starting point from which the shock intensities will be varied for each animal.

General activity is measured for 30 minutes in a 40 cm X 40 cm X 30 cm clear plexiglas chamber utilizing a grid of photo beams. Horizontal and vertical activity (number of beam breaks) is measured in 10-min segments along with total activity for the 30-min session. Habituation is being defined as the decline in activity as a function of time during the session [14] (results not shown).

Animal Care and Handling: Research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 1996. The facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

TABLE 1. General Experimental Scheme.

Implant Electrode	Baseline	Chronic Exposure	Post-Exposure					
All Ss	2 days	2 weeks/M-F	2 hrs	3	10	30	100 days	
	(EEG, Behavior)	(EEG, Behavior)	(EEG, Behavior, Neurochem, Histology)					Animal # Subtotal
		Saline	6, 6	6, 6	6, 6	6, 6	6, 6	60
		Sarin: 0.2 LD50	6, 6	6, 6	6, 6	6, 6	6, 6	60
		Sarin: 0.4 LD50	6, 6	6, 6	6, 6	6, 6	6, 6	60
								180 Total

Two groups of 6 animals at each time point: six are being tested for flinch threshold and six for activity level. Animals from both behavioral groups are being used for ChE and receptor assays, and neuropathological evaluation.

RESULTS

The present report represents interim results of this ongoing study. Since within a dose condition, all animals are treated the same during the exposure phase, there are sufficient animals to determine any obvious trends that may have developed during this phase of the study (See figure legends for Ns).

During the daily post-exposure EEG recording period (1 hr), there were no observable signs of sarin exposure on behavioral and sensory indices: eyelid closure, writhing, vocalization, circling, biting, and the ease of handling, lacrimation and salivation. For further discussion of the results, see Figures 1-7:

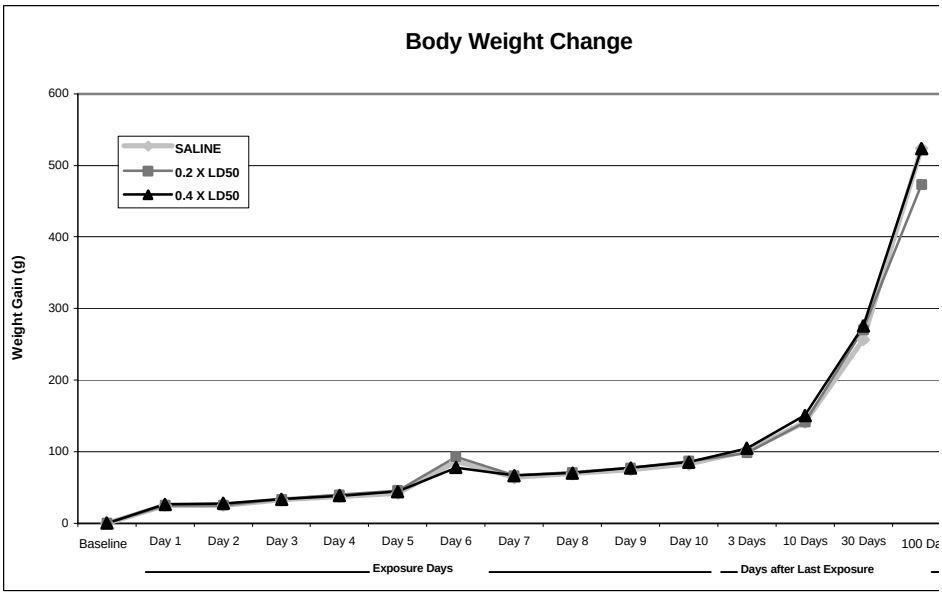

Figure 1. Body Weight Change**:** There was no difference in body weight gain (g) between saline and either 0.2 or 0.4 X LD50 sarin animals over the 2-week exposure period or up to 100 days post-exposure. (Ns = 20-23 animals for all three groups from initial the baseline day throughout the exposure period, however, at 3 days after the last exposure, Ns = 16-19; at 10 days, Ns = 11-14; at 30 days, Ns = 9; and at 100 days, Ns = 2-3.)

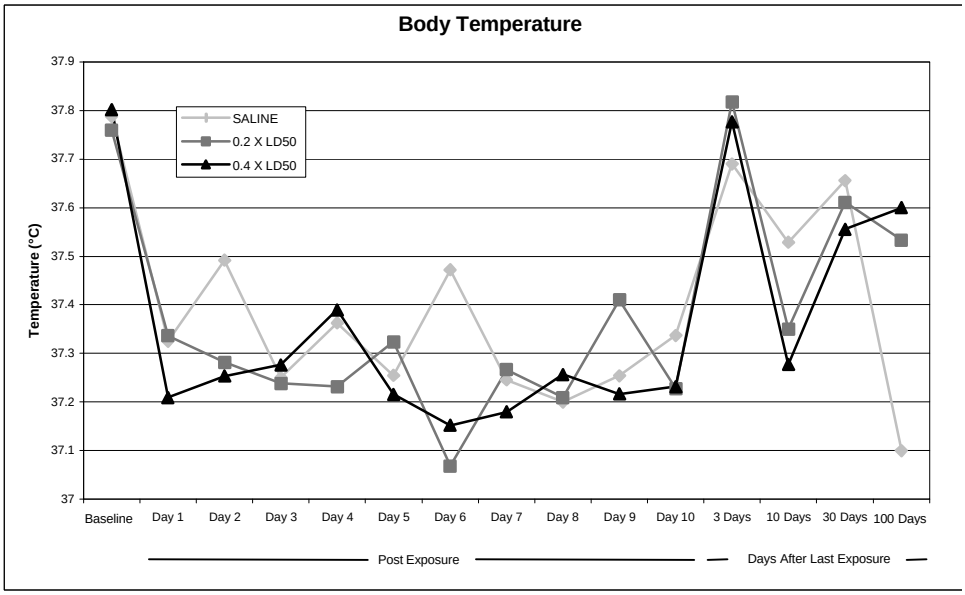

Figure 2. Body Temperature**:** There was no significant difference in body temperature (ºC) between saline and either 0.2 or 0.4 X LD50 sarin animals. Temperature shown on exposure days 1-10 was taken 1 hour post-injection. All temperature measures in this portion of the study were taken using a rectal probe (YSI Thermometer). (Ns = same as for Body Weight, see Figure 1.)

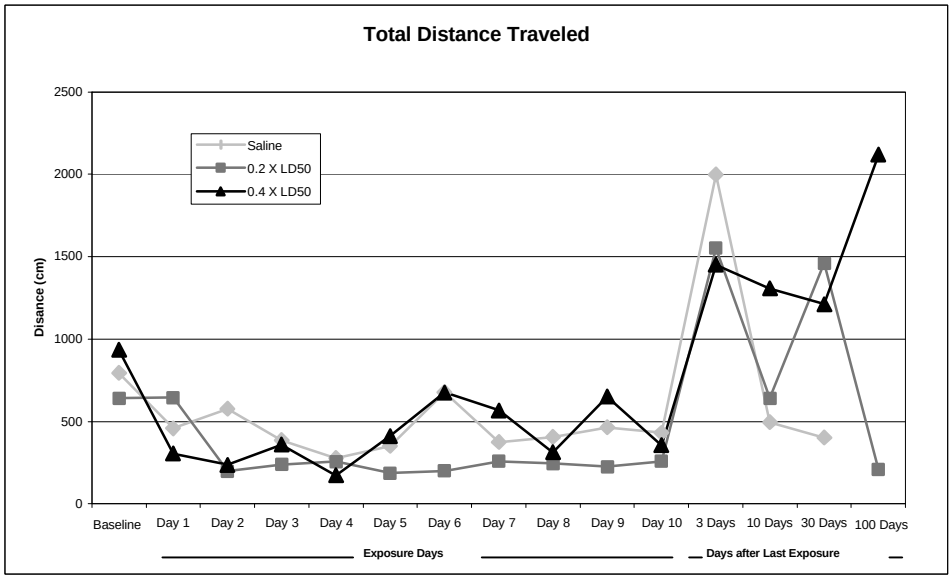

Figure 3. Total Distance Traveled (Activity Level): There was no effect of dose on activity level, stated as a function of distance traveled (cm). The large jump in activity level observed in all three groups at 3 days after the last exposure is likely due to the weekend break and the absence of the injection handling. Additionally, animals are present in the EEG chamber (prior to activity testing) for only 15 minutes, compared to 1 hr, 15 min on injection days. (Ns = same as for Flinch Threshold (see Figure 5), except for the 100-day time point in which (at this point in the study) Ns = 1 for the saline and 0.4 LD50 groups, and N = 0 for the 0.2 LD50 group.)

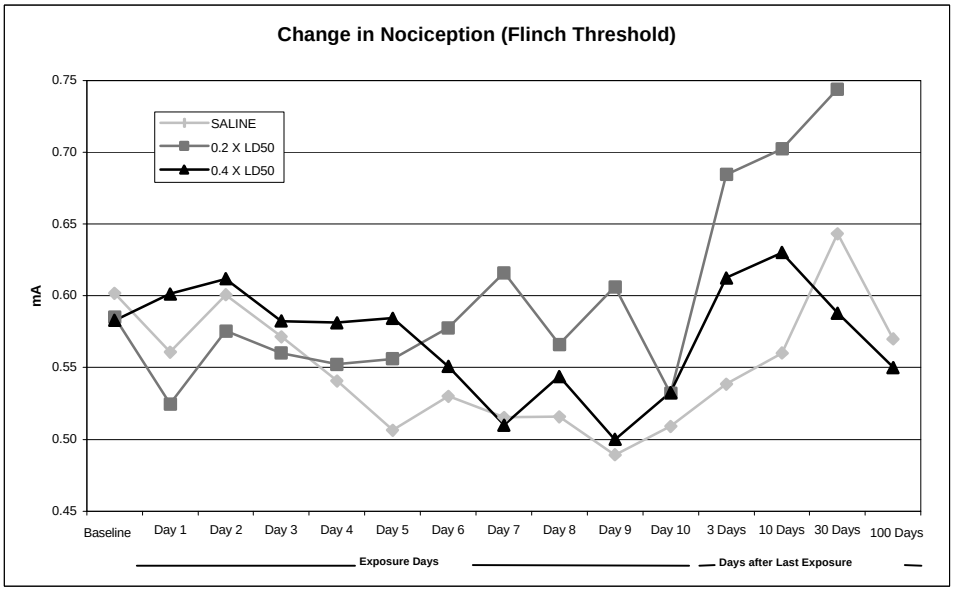

Figure 4. Change in Nociception (Flinch Threshold)**:** There was no meaningful effect of sarin dose on a measure of nociception (flinch threshold). Shown are raw flinch thresholds (mA) for pre-exposure period baseline, exposure days 1-10 (measured 1 hr post-injection), and selected days after the exposure period. (Ns = 9-16 for all three groups for baseline through exposure day 10; however, at 3 days after the last exposure, Ns = 6-11; at 10 days, Ns = 6-9; at 30 days, Ns = 3-6; and at 100 days, Ns = 1-2.)

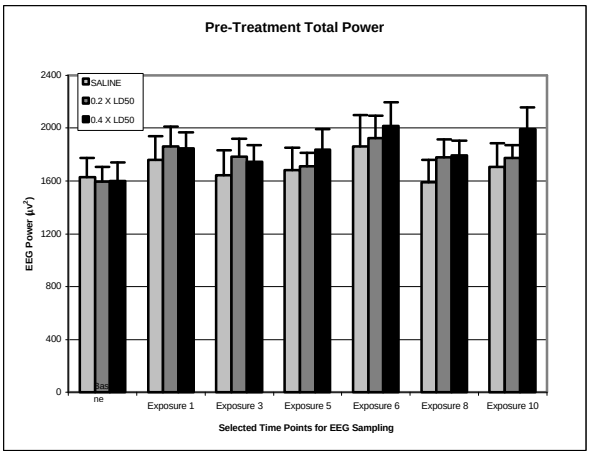

Figure 5. Pre-Treatment Total EEG Power**:** There was no significant effect of either the 0.2 or 0.4 LD50 sarin dose on total EEG power ($ìv^2$), when pre-exposure period baseline EEG was compared with the 10-day exposure period pre-injection EEG recordings ($F_{12,6}$ = 0.60, P> 0.83). (For Figures 3 and 4, N = 11 (saline); N = 12 (0.2 LD50), N = 13 (0.4 LD50.)

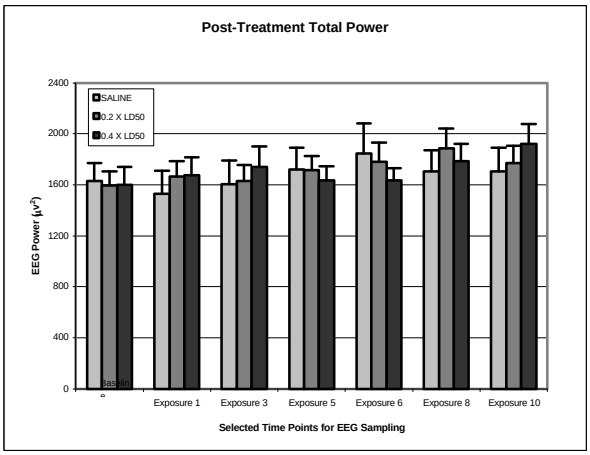

Figure 6. Post-Treatment Total EEG Power**:** There was no significant effect of either the 0.2 or 0.4 LD50 sarin dose on total EEG power ($ìv^2$), when the pre-exposure period baseline EEG measures were compared with exposure period daily post-injection EEG recordings ($F_{12,6}$ = 1.035, P> 0.43). (On both pre- and post-treatment EEG, analysis of the five individual bands (spectral analysis: ä, è, á, $â_1$, $â_2$) also resulted in no significant effect of sarin dose on individual band power.)

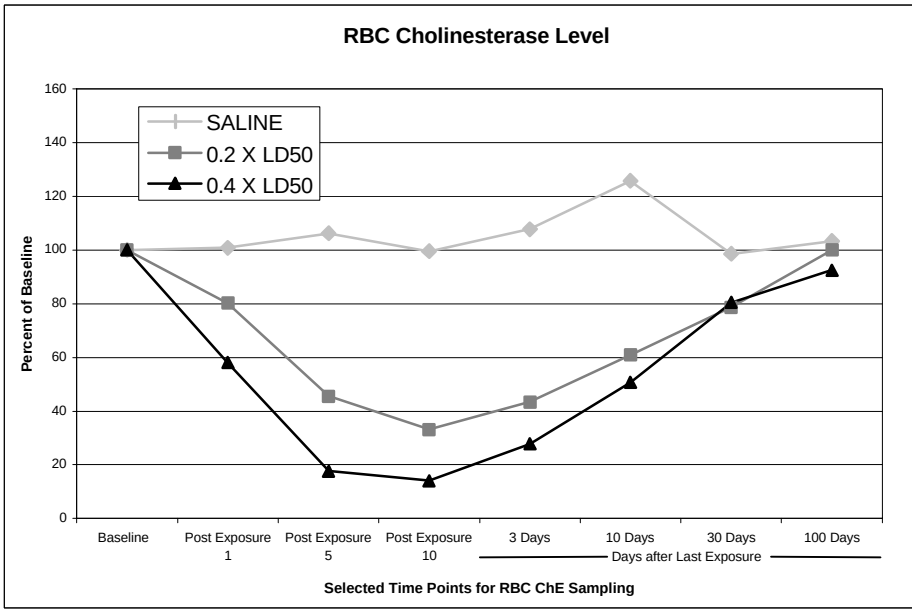

Figure 7. RBC Cholinesterase Level: In contrast to the absence of behavioral or EEG signs, RBC ChE activity dropped to less than 20% of baseline following the tenth exposure in the 0.4 LD50 sarin animals, and to less than 40% of baseline in the 0.2 LD50 animals. Both agent groups showed a steady increase in ChE activity following the exposure period, with 0.2 animals returning to baseline and 0.4 animals remaining just below baseline at 100 days after the last exposure.

CONCLUSIONS

It is important to reiterate that these results are preliminary, since the study is ongoing. While it is informative to gain an idea of the direction the results may be taking, drawing definitive conclusions at this point would be premature. Further, the receptor binding and brain ChE activity assays and the histopathological analyses are currently being carried out; consequently those data sets are insufficiently complete to be presented here.

However, during the pre-exposure period baseline and exposure day pre- and post-injection measures, the N's are sufficiently large (~20) on a number of parameters to allow speculation. So far, we have observed no behavioral indication of an effect of low-dose sarin exposure, at least at the doses and schedule used here. However, the drop of RBC ChE activity to below 20% of baseline in the 0.4 LD50 sarin group, and to below 40% of baseline in the 0.2 LD50 group, represents a dramatic contrast. How this change in ChE activity will affect receptor binding, brain ChE activity and neuropathology is not known. Nevertheless, the drop to 40% and 20% of baseline ChE activity is sufficient to suggest that alterations in intracellular parameters--either biochemical or histopathological--will be observed in the sarin-exposed animals, at least transiently (at the earlier time points) and perhaps persistently (at 30 and 100 days post-exposure). When the study is complete, it may be possible to connect these suggested neurochemical and/or neuropathological alterations with some of the adverse health effects reported by Gulf War veterans.

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