



U.S. ARMY MEDICAL RESEARCH INSTITUTE OF CHEMICAL DEFENSE

USAMRICD-TR-03-04

Scopolamine Antagonizes the Lethal Effects
of O-isobutyl S-[2-(diethylamino)ethyl]-
methylphosphonothioate (VR)

Fat-Chun T. Chang
Sandra J. DeBus

May 2003

20031103 036

Approved for public release; distribution unlimited

U.S. Army Medical Research
Institute of Chemical Defense
Aberdeen Proving Ground, MD 21010-5400

DISPOSITION INSTRUCTIONS:

Destroy this report when no longer needed. Do not return to the originator.

DISCLAIMERS:

The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or as reflecting the views of the Army or the Department of Defense.

In conducting the research described in this report, the investigators complied with the regulations and standards of the Animal Welfare Act and adhered to the principles of the Guide for the Care and Use of Laboratory Animals (NRC 1996)."

The use of trade names does not constitute an official endorsement or approval of the use of such commercial hardware or software. This document may not be cited for purposes of advertisement.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour 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) 2003		2. REPORT TYPE Technical Report		3. DATES COVERED (From - To) January 2000 to October 2001	
4. TITLE AND SUBTITLE Scopolamine Antagonizes the Lethal Effects of O-isobutyl S-[2-(diethylamino)ethyl]methylphosphonothioate (VR)				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER 62384	
6. AUTHOR(S) Chang, F-C.T., DeBus, S.				5d. PROJECT NUMBER TC2	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) US Army Medical Research Institute of Chemical Defense ATTN: MCMR-UV-PA 3100 Ricketts Point Road Aberdeen Proving Ground, MD 21010-5400				8. PERFORMING ORGANIZATION REPORT NUMBER USAMRICD-TR-03-04	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) US Army Medical Research Institute of Chemical Defense ATTN: MCMR-UV-RC 3100 Ricketts Point Road Aberdeen Proving Ground, MD 21010-5400				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					
14. ABSTRACT O-isobutyl S-[2-(diethylamino)ethyl]methylphosphonothioate (VR) causes cholinergic hyperfunctions, seizures, convulsions, cardiorespiratory failure, and death. This study evaluated the effectiveness of scopolamine in antagonizing VR-induced pathophysiology and lethality in guinea pigs chronically instrumented for concurrent recordings of electrocorticogram, diaphragm electromyogram, Lead II electrocardiogram and neck skeletal muscle electromyogram. Thirty min prior to intoxication with a 2xLD ₅₀ of VR (22.6 µg/kg, sc;), guinea pigs were pretreated with pyridostigmine (0.026 mg/kg, im). Within 1 min after VR intoxication, they were treated with 2-PAM (25 mg/kg, im) and scopolamine (0.1, 0.25 or 0.5 mg/kg, im). All animals survived the VR challenge 24 hr later; none displayed seizures or convulsions. In animals receiving 0.1 mg/kg scopolamine, however, 33% developed a brief period (3-5 min) of elevated cortical excitability. Scopolamine was also remarkably effective in reversing VR-induced cholinergic hyperfunctions and cardiorespiratory dysfunctions. Restoration of cardiorespiratory functions following scopolamine was prompt: With the exception of transient periods of mild tachycardia and tachypnea, the cardiorespiratory activity profiles of all three scopolamine dose groups appeared normal throughout the course of intoxication and recovery. Findings from this study showed that a higher scopolamine dose (0.25 or 0.5 mg/kg) seemed to provide much better protection against VR-induced CNS excitability and cardiorespiratory dysfunctions.					
15. SUBJECT TERMS O-isobutyl S-[2-(diethylamino)ethyl]methylphosphonothioate (VR), respiration, cardiovascular function, seizures, novel chemical warfare agent, VX isomer, scopolamine, acetylcholinesterase inhibitor, guinea pigs					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UNLIMITED	18. NUMBER OF PAGES 18	19a. NAME OF RESPONSIBLE PERSON Fat-Chun T. Chang
a. REPORT UNCLASSIFIED	b. ABSTRACT UNCLASSIFIED	c. THIS PAGE UNCLASSIFIED			19b. TELEPHONE NUMBER (include area code) 410-436-1343

ABSTRACT

O-isobutyl S-[2-(diethylamino)ethyl]methylphosphonothioate (VR), an extremely toxic organophosphorus compound, is known to cause cholinergic hyperfunctions, seizures, convulsions, cardiorespiratory failure, and death (Chang *et al.*, 1998). In this study, the effectiveness of scopolamine in antagonizing VR-induced pathophysiology and lethality was evaluated in guinea pigs chronically instrumented for concurrent recordings of electrocorticogram (ECoG), diaphragm electromyogram (DEMG), Lead II electrocardiogram (ECG_{II}) and neck skeletal muscle electromyogram (NEMG). Thirty (30) min prior to intoxication with a 2xLD₅₀ of VR (22.6 µg/kg, sc;), the guinea pigs were pretreated with pyridostigmine (0.026 mg/kg, im). Within 1 min after VR intoxication, the animals were treated with 2-PAM (25 mg/kg, im) and scopolamine (0.1, 0.25 or 0.5 mg/kg, im). All animals survived the VR challenge 24 hr later. None of the animals of three scopolamine dose groups displayed seizures or convulsions. In animals receiving 0.1 mg/kg scopolamine, however, 33% developed a brief period (3-5 min) of elevated cortical excitability. Scopolamine was also remarkably effective in reversing VR-induced cholinergic hyperfunctions and cardiorespiratory dysfunctions. Restoration of cardiorespiratory functions following scopolamine was prompt: With the exception of transient periods of mild tachycardia and tachypnea, the cardiorespiratory activity profiles of all three scopolamine dose groups appeared normal throughout the course of intoxication and recovery. In summary, findings from this study showed that while lethality can be averted with only 0.1 mg/kg scopolamine, a higher scopolamine dose (e.g., 0.25 or 0.5 mg/kg) seemed to provide much better protection against VR-induced CNS excitability and cardiorespiratory dysfunctions.

INTRODUCTION

O-isobutyl S-[2-(diethylamino)ethyl]methylphosphonothioate (VR) is a recently disclosed novel chemical warfare agent from Russia (Szafraniec *et al.*, 1995). VR, an extremely potent acetylcholinesterase (AChE) inhibitor (Maxwell *et al.*, 1997), is a structural isomer of a more widely known organophosphorus (OP) chemical warfare agent, O-ethyl S-[2(diisopropylamino)-ethyl]methylphosphonothioate (VX). VR is extremely toxic. Maxwell and co-workers (1997) have shown that the guinea pig LD₅₀ of VR (11.3 µg/kg, sc; range, 9.9-12.6 µg/kg) was quite similar to that of VX (8.9 µg/kg, sc; range, 7.6-10.2 µg/kg).

Acute exposure to a lethal dose of VR in guinea pigs has been shown to cause progressive cardiorespiratory depression and death (Chang *et al.*, 1998; 2002; Maxwell *et al.*, 1997). Experimental therapeutics investigations (Chang *et al.*, 2002; Maxwell *et al.*, 1997; Shih and McDonough, 2000) showed that VR-induced lethality and other life-threatening cardiorespiratory symptoms can be antagonized with a regimen involving carbamate pretreatment and oxime/atropine treatment (Dirnhuber *et al.*, 1979; Dunn and Sidell, 1989; Keeler *et al.*, 1991; Leadbeater *et al.*, 1985). While this regimen was effective in preventing lethality, it had little, if any, impact on the development and progression of seizures unless an anticonvulsant adjunct (e.g. diazepam) was also given following exposure. Further evaluation of this pretreatment/therapy regimen revealed that, all else being equal, increases in atropine dose levels (from 2 mg/kg to 8 or 16 mg/kg) can, in an incrementally more effective manner, block VR-induced seizures and restore a variety of aberrant cardiorespiratory activities to a level comparable to that of control (Chang *et al.*, 2002). OP-induced seizure activity is an important medical management issue since sustained periods of uncontrolled cortical excitability can cause brain injury and prolonged physical incapacitation (Churchill *et al.*, 1985; Lallement *et al.*, 1991; 1993; 1994; Lemerrier *et al.*, 1983; McLeod, 1985; McDonough *et al.*, 1989; Petras, 1994). Currently, diazepam (10 mg intramuscular autoinjector) is made available to the U.S. military personnel for controlling seizures and/or convulsions in the combat environment. Notwithstanding, the extent of CNS protection offered by diazepam has recently been questioned (Baze, 1993; Clement and Broxup, 1993; Hayward *et al.*, 1990; Martin *et al.*, 1985; McDonough *et al.*, 1995; Phillipens *et al.*, 1992) and anticonvulsant compounds other than benzodiazepine derivatives are currently being sought and evaluated.

Based on findings derived from an earlier investigations (Chang *et al.*, 1998), we began to focus our attention on scopolamine as a potential alternative for antagonizing VR-induced seizures. Scopolamine (hyoscine) is a belladonna alkaloid that possesses anticholinergic activities similar to those of atropine. The potential of scopolamine as an antidote for OP poisoning has been the subject of investigation in this laboratory for many years (Capacio and Shih, 1991; Harris *et al.*, 1994; Lennox *et al.*, 1992; Solana *et al.*, 1991). Anderson and co-worker (1994; 1997) argued further that scopolamine could possibly replace atropine or diazepam, or both, as a therapeutic compound against soman toxicities. The purpose of this study is to examine more closely the effectiveness of scopolamine in antagonizing VR-induced seizures, convulsions, cardiorespiratory dysfunctions and death.

METHODS

Surgical Instrumentation. Eighteen (18) barrier raised male Hartley albino guinea pigs (*Cavia porcellus*) weighing between 550-1010 g were used in this study. Each animal was surgically instrumented for concurrent electrophysiological recordings of i) electrocorticogram (ECoG); ii) diaphragmatic electromyogram (DEMG); iii) neck skeletal muscle electromyogram (NEMG); and iv) Lead II electrocardiogram (ECG_{II}; from which heart rates could be derived). ECoG was monitored to evaluate changes in CNS excitability (such as seizures) in response to VR and pretreatment/therapy. DEMG activity recordings were made to provide information concerning the animal's ventilatory status as well as the extent of perturbation and recovery in diaphragmatic function throughout the course of VR intoxication and therapy. Neck skeletal musculature (NEMG) activities were documented to assess the extent of aberrant changes in skeletal muscle activity (such as fasciculation). Finally, ECG_{II} signals and heart rates were surveyed to reveal changes in general cardiovascular status of the animal and the functional integrity of the myocardium throughout the course of VR intoxication and therapy. Methods of surgery and chronic instrumentation procedures for electrophysiological recordings of diaphragmatic electromyogram, ECoG, ECG_{II} and NEMG have been documented elsewhere (Chang and Harper, 1989; Chang *et al.*, 1998). Readers are referred to the reports cited above for technical details.

Intoxicant. Stock solutions of O-isobutyl S-[2-(diethylamino)ethyl]methylphosphonothioate (VR; 1 mg/ml) were obtained from the chemical surety materials facility of the U.S. Army Medical Research Institute of Chemical Defense. Working solution (45.2 µg/ml) of VR was prepared from stock aliquots minutes before intoxication. In this study, each animal was intoxicated with a 2-LD₅₀ VR dose (22.6 µg/kg, sc; dose volume, 0.5 ml/kg).

Pretreatment and Therapy. Each animal was pretreated with pyridostigmine hydrobromide (0.026 mg/kg, im; dose volume, 0.25 ml/kg) thirty (30) minutes prior to VR intoxication. Seconds after VR administration, a therapy admixture (dose volume, 0.25 ml/kg; im) of scopolamine hydrobromide (0.1, 0.25 or 0.5 mg/kg) and 2-PAM chloride (25 mg/kg) was given intramuscularly.

Experimental Group Design. Animals (n=18) were randomly assigned to three (3) experimental groups (n=6 per group). All animals received the pyridostigmine pretreatment followed by VR intoxication and a therapy mixture of scopolamine and 2-PAM. For Groups 1, 2 and 3, the scopolamine dose levels in the therapy mixture were 0.1 mg/kg, 0.25 mg/kg, and 0.5 mg/kg respectively.

Electrophysiological Recording. Each animal served as its own control. Cardiorespiratory activity profile and other physiological variables were continuously monitored and recorded throughout the course (≈6-8 h) of intoxication and recovery. A final 15-min recording was made 24 h after VR intoxication and pretreatment/therapy.

ECoG, DEMG and NEMG signals were amplified and band-pass filtered (DEMG and NEMG, 50-7,500 Hz; ECoG, 0.5-500 Hz). All physiological activities and experimental events were recorded with a multi-channel FM analog tape recorder.

Data Analysis. Data analyses were performed off-line. Electrophysiological data were analog-to-digital converted and analyzed in accordance with the following scheme.

1. Power Spectral Analyses of DEMG and ECoG Activities. Power spectral analyses (Childers, 1978) were performed to evaluate the extent of changes in DEMG and ECoG activities. DEMG data were sampled at a rate of 5 KHz for 32.768 sec (20 epochs; 8192 points/epoch). ECoG data were sampled at a rate of 625 Hz for 32.768 sec (20 epochs; 1024 points/epoch). "Zero Mean" was applied to both DEMG and ECoG data. The running sums of DEMG data were "cosine-tapered" before Fast Fourier Transforms were computed. DEMG spectra were smoothed with a 15-point polynomial filter.

2. ECG_{II} and heart rate data were normalized and expressed graphically as percentage of control \pm standard error of the mean (SEM). Wherever appropriate, unpaired t-tests were performed to assess the effect of VR and pretreatment/therapy on cardiorespiratory activity patterns.

3. For clarity of data presentation, we have classified CNS and cardiorespiratory changes throughout the course of VR intoxication and pretreatment/therapy into control stage and four (4) experimental stages. The experimental stages were temporally designated as Post-VR Stages at i) +20-min, ii) +2-hr, iii) +6-hr, and iv) +24-hr.

RESULTS

The first signs of VR intoxication (ca. 3-7 min post-VR) began with oro-facial movements indicative of excessive mucoid/salivary hypersecretion and heightened alerting responses to novel sensory (auditory, visual, tactile) stimuli. As the toxicity progressed the animals started to exhibit mild tremors/fasciculations and loss of righting reflexes. The symptoms mentioned above were consistently seen in Group 1 animals (0.1 mg/kg scopolamine) with abating severity for about 2 h post-VR. These symptoms were much less pronounced in Group 2 (0.25 mg/kg scopolamine) animals. Tremors and fasciculations were absent in Group 3 (0.5 mg/kg) animals, although hyper-arousal and hypersecretion could still be observed. Within about 1-2 h post-VR, Groups 2 animals were able to resume normal behavior and appeared symptom-free. Hyper-arousal and hypersecretion disappeared completely in about half an hour. Parenthetically, more severe cholinergic hyperfunctions typically associated with VR intoxication, such as bradycardia, bradypnea, seizures, convulsions, etc. (see Chang *et al.*, 1998; 2002), were not observed in any of the animals across Groups 1-3.

Figure 1 (panels A-C) is an electrophysiographic depiction of CNS and cardiorespiratory responses throughout VR intoxication and recovery. Cardiorespiratory changes were noticeable only as modest increases in respiratory frequency and heart rate during the first 2-3 h after VR (see Fig. 4; *vide infra*). The increase in cardiorespiratory activity profile can be attributable to vagally mediated anticholinergic effects following scopolamine administration. Brief periods of elevated cortical excitability lasting about 3-5 min were noted in 33% of Group 1 animals (0.1 mg/kg scopolamine). No aberrant CNS activities (such as seizures) were noted in Groups 1-3 animals throughout the course of intoxication and recovery. Irrespective of the scopolamine dose levels, all animals recovered from a 2xLD₅₀ VR challenge and survived 24 h later with no indications of neurobehavioral abnormalities.

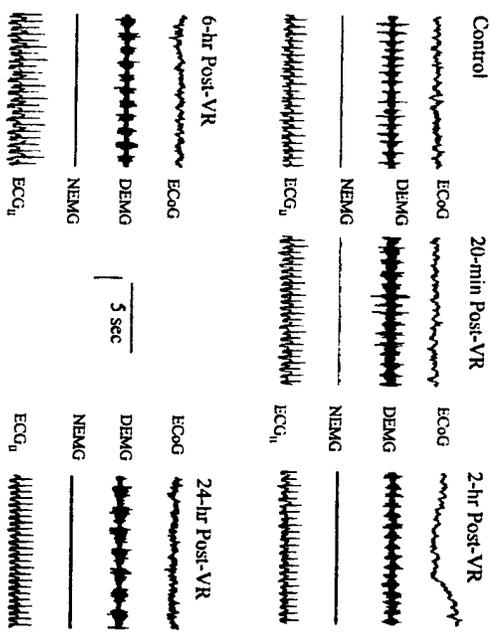


Figure 1A
0.1 mg/kg Scopolamine

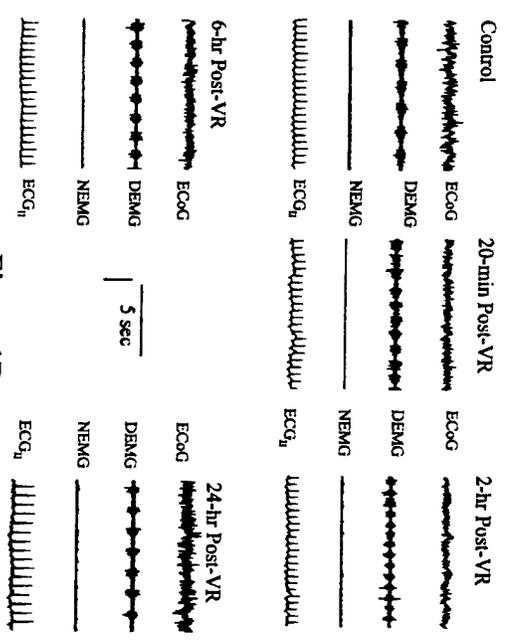


Figure 1B
0.25 mg/kg Scopolamine

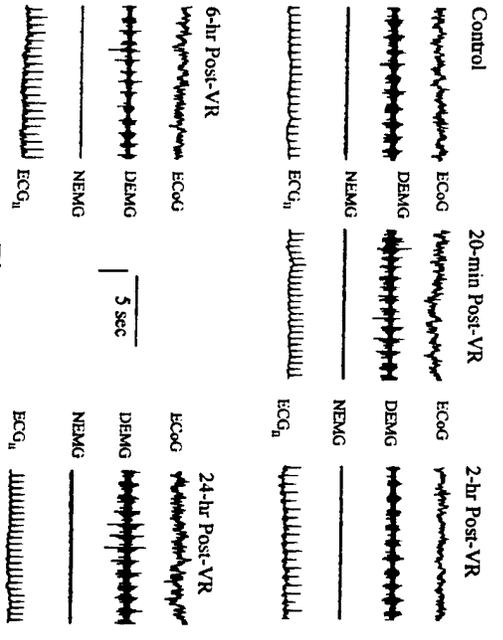


Figure 1C
0.5 mg/kg Scopolamine

Figure 1 (A-C). An electrophysiological depiction of VR-induced pathophysiology and responses to pretreatment and therapy of three representative animals from Groups 1-3. With the exception of varied scopolamine doses (*vide infra*), all animals were pretreated with pyridostigmine (0.026 mg/kg, *im*), intoxicated with VR (2xLD50 or 22.6 μ g/kg), and given 2-PAM (25 mg/kg, *im*) for acetylcholinesterase reactivation. The scopolamine doses were: Group 1 (Fig. 1A), 0.1 mg/kg; Group 2 (Fig. 1B), 0.25 mg/kg; and, Group 3, 0.5 mg/kg. Note that i) seizure development was blocked across all three scopolamine dose groups, and ii) the cardiorespiratory profiles did not appear to be dysfunctional throughout the course of intoxication and treatment. Signal trace description: ECoG, electrocorticogram; DEMG, diaphragmatic electromyogram; NEMG, electromyographic recording from the neck muscle; and ECG_{II}, Lead II electrocardiogram. Voltage calibrations: ECoG, 100 μ V; DEMG, 1.34 mV; NEMG, 1.17 mV.

In an earlier investigation, we have shown that atropine (2, 8 or 16 mg/kg, co-administered with 2-PAM in pyridostigmine-pretreated guinea pigs) was effective in antagonizing VR-induced lethality (Chang *et al.*, 2002). More specifically, at 2 mg/kg, atropine was only effective in preventing lethality, but not seizures, convulsions and cardiorespiratory depression. When the dose level was increased to 8 mg/kg, atropine was capable of blocking seizures/convulsions in only 50% of the animals and offered marginal protection to cardiorespiratory functions. Only at the dose level of 16 mg/kg was atropine able to block the development of seizures/convulsion, cardiorespiratory dysfunctions, and prevent lethality. The overall effectiveness of atropine pales, however, in comparison to scopolamine. As data in this report indicate, scopolamine could consistently bring about a complete symptomatic reversal at a dose of only 0.1 mg/kg. Also notable were the duration and intensity of cholinergic symptoms following either atropine or scopolamine treatment. That is, animals treated with 0.1 mg/kg scopolamine were able to right and began to engage in normal behavioral repertoires within 2 h post-VR. In guinea pigs treated with 16 mg/kg atropine, it took more than 4 h for the animals to restore their righting reflexes and resume normal behavioral repertoire.

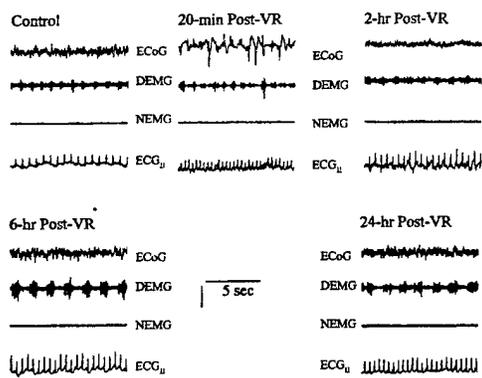


Figure 2
8 mg/kg Atropine (Seizure)

Figure 2. VR-induced pathophysiology and responses to pyridostigmine pretreatment and therapy with 2-PAM and atropine (8 mg/kg). One half (50%) of animals receiving 8 mg/kg atropine developed seizures (see the top middle panel labeled +20-min Post-VR), which were subsequently brought under control with diazepam (see panels labeled 2, 6 and 24-h Post VR). Parenthetically, the other half of animals receiving 8 mg/kg atropine and all of the animals receiving 16 mg/kg atropine showed no sign of seizure activity (Chang *et al.*, 2002). Voltage calibrations: ECoG, 100 μ V; DEMG, 1.44 mV; NEMG, 1.01 mV.

Figure 2, for the purpose of comparison, is an electrophysiographic depiction of CNS responses and cardiorespiratory activity profiles throughout the course of VR intoxication and treatment with 8 mg/kg atropine (and 2-PAM) in a pyridostigmine-pretreated animal.

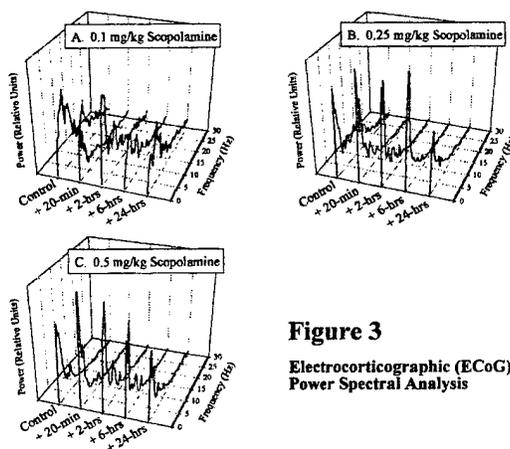


Figure 3
Electrocorticographic (ECoG)
Power Spectral Analysis

Figure 3 (A-C). Power spectral analysis of electrocorticographic (ECoG). Results shown here demonstrated that scopolamine was able to effectively block the development of VR-induced seizures with any of the three scopolamine doses used in this study.

Electrocorticographic (ECoG) Responses. All animals (Groups 1-3) became increasingly restless and aroused by VR and scopolamine during the first 10-15 min post-intoxication. Concurrent with the development of "alerting behaviors" was the transformation of a wakefulness, resting ECoG patterns into to an "arousal" profile characterized by i) a small but notable reduction in the amplitudes of a low frequency (0.5-4 Hz) power spectral complex and ii) an increase in the amplitudes of a 5-20 Hz ECoG power spectral complex. The "alerting/arousal" ECoG profile typically continued with abating severity for about 5-15 min and eventually returned to that of control characteristics. In 33% (n=2) of the Group 1 animals (0.1 mg/kg scopolamine), the "alerting" ECoG pattern was transformed into a brief period (3-5 min) of augmented cortical excitability. The elevated cortical excitability pattern was characterized by a 1-2 Hz oscillatory power spectral pattern with an amplitude approximately 2-3 times that of the normal wakefulness ECoG pattern. Figure 3 (panels A, B and C) is a power spectrographic depiction of ECoG responses to VR intoxication and therapy. CNS protection by scopolamine was quite striking: Throughout the post-treatment and recovery periods, there was no sign of seizures or convulsions.

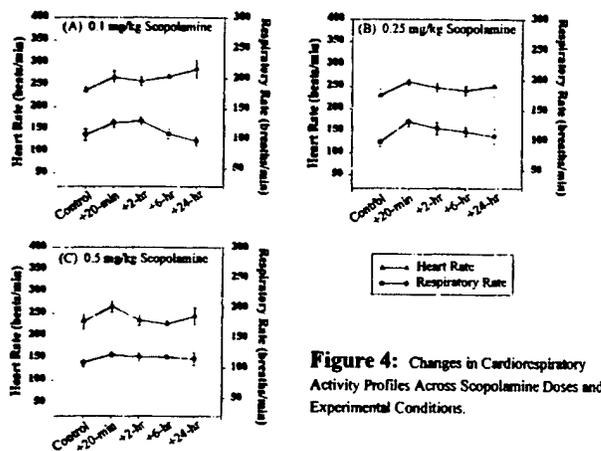


Figure 4: Changes in Cardiorespiratory Activity Profiles Across Scopolamine Doses and Experimental Conditions.

Figure 4. Changes in respiratory rate (filled circles) and heart rate (filled triangles) in response to VR and pretreatment/therapy regimen with 0.1 mg/kg (Fig. 4A), 0.25 mg/kg (Fig. 4B) and 0.5 mg/kg (Fig. 4C) scopolamine. Note a consistent, albeit statistically insignificant, increase in heart rate during the first 2-3 h following VR/scopolamine. Unpaired t-tests were performed to assess the effect of VR and pretreatment/therapy on cardiorespiratory activity patterns and none of the post-VR/scopolamine data points was found to be significantly different from those of control condition. Error bar = Standard Error of the Mean (SEM).

Cardiorespiratory Responses. Changes in heart rate (filled triangles) and respiratory rate (filled circles) throughout the course of VR intoxication and therapy are depicted in Figure 4.

1. **Heart Rate and ECG_{II} Waveform Attributes.** Heart rate typically showed an increase for 2-3 h after scopolamine in all three experimental groups (Figs. 4A, 4B and 4C; filled triangles). The magnitude and duration of scopolamine-induced increase in heart rate did not appear to be dose-dependent over the dose range used in this study. A somewhat intriguing phenomenon consistently seen in animals across all three scopolamine dose groups was a higher than control level heart rate 24 h after VR intoxication. Examination of waveform attributes of ECG_{II} records throughout intoxication and recovery did not reveal any signs of myocardial abnormalities (such as bradycardia, QT-prolongation, arrhythmias, J-Point elevation, T-inversion, etc.) that were typically seen in OP intoxication.

2. Respiratory Rate and Pattern. A tachypneic profile (increase in respiratory frequency) was seen during the first 20-30 min of VR intoxication in all three groups (see Figs. 3A, 3B and 3C; filled circles). Subsequent changes in respiratory rate across three experimental groups varied somewhat. Generally speaking, irrespective of scopolamine dose levels, the respiratory patterns began to show behavior- or state-dependent modulations about 0.5-1 h after scopolamine. The ability of respiratory system components to undergo modulation with behavior and states of consciousness indicated the full return of central respiratory functional integrity as well as resumption of a functionally dynamic central respiratory mechanism. The respiratory frequency across all three dose groups was returned to a level comparable to that of control 2-3 h following scopolamine treatment. A statistically insignificant 5-10% respiratory rate depression, however, was seen in about 50% of Group 1 animals at +24 hr post-VR. For Group 2 and 3 animals, the respiratory frequency either showed a return to control level or a slight increase at +24 hr post-VR. Further examination of control respiratory frequency of Group 2 and 3 animals indicated that the increase or decrease in respiratory rates at the +24-hr stage was well within the normal limits of variability and was therefore statistically inconsequential.

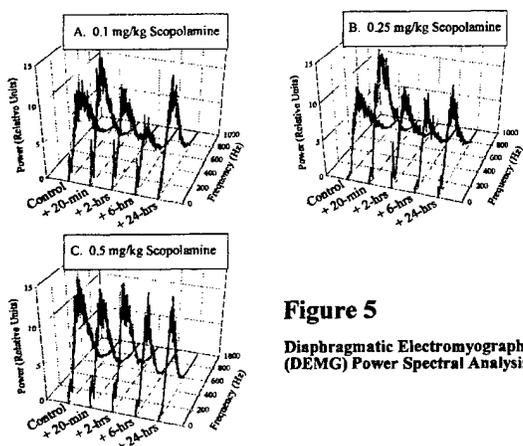


Figure 5
Diaphragmatic Electromyographic (DEMG) Power Spectral Analysis

Figure 5. Power spectrographs showing changes in the amplitude of diaphragmatic activity in response to VR across i) 0.1 mg/kg (Fig. 5A), ii) 0.25 mg/kg (Fig. 5B), and iii) 0.5 mg/kg (Fig. 5C) scopolamine. Note that animals receiving 0.1 mg/kg and 0.25 mg/kg scopolamine all appeared to show a slight increase in DEMG amplitudes at the +20-min post-VR stage. The augmentation in the spectral power of DEMG activities was typically followed by a period of small DEMG amplitude depression (see +2 and 6-hr post-VR stages; Figs. 5A and 5B). Changes in DEMG spectrographic attributes in animals receiving 0.5 mg/kg scopolamine (Fig. 5C) were not particularly noteworthy.

3. Diaphragmatic Responses. Figure 5 is a power spectrographic depiction of VR-induced changes in DEMG activities throughout intoxication and recovery. For Groups 1 and 2, the spectral powers of diaphragmatic EMG were notably augmented for about 40-80 min after VR/scopolamine (see +20-min post-VR stage; Figs. 5A and 5B). In Group 1 animals, the initial augmentation was followed by a gradual reduction in spectral power (see +6-hr post-VR stage; Fig. 5A). For Group 2 animals, the DEMG power typically showed a return to control levels 1-2 h following scopolamine. Changes in DEMG spectral powers in Group 3 animals were not particularly remarkable throughout the 24-h period.

Closer examination of DEMG power spectrographs further revealed that changes (increase or decrease) in DEMG amplitudes in Group 1 and 2 animals seemed to involve the entire frequency

spectrum (1-1000 Hz). Moreover, the amplitude changes were not limited to, or associated with, any particular spectral range/components. Finally, despite variations in respiratory rate and discrete spectral powers, the temporal attributes of respiratory pattern throughout the course of intoxication and recovery remained virtually unchanged across animals receiving 0.1 mg/kg (Group 1), 0.25 mg/kg (Group 2), or 0.5 mg/kg (Group 3) scopolamine.

DISCUSSION

The antidotal effectiveness of an experimental therapeutics regimen consisting of pyridostigmine pretreatment and scopolamine/2-PAM therapy against a lethal dose ($2xLD_{50}$) of VR was described in this report. Animals from all three scopolamine dose groups (0.1, 0.25 and 0.5 mg/kg) survived the $2xLD_{50}$ VR challenge 24 hours later with no signs of neurobehavioral abnormality. Electrophysiological records taken throughout the course of intoxication and recovery revealed only a modest increase in respiratory rate and heart rate (attributable primarily to the anticholinergic properties of scopolamine) following scopolamine treatment. The extent of scopolamine-mediated CNS protection was quite striking: With the exception of a brief period of elevated cortical excitability seen in 33% of Group 1 (0.1 mg/kg) animals shortly after VR, none of the animals from the three scopolamine dose groups developed seizures or convulsions.

We have shown in an earlier investigation (Chang *et al.*, 2002) that guinea pigs pretreated with pyridostigmine and subsequently treated with atropine (2 or 8 mg/kg) and 2-PAM were able to survive a $2xLD_{50}$ VR challenge. This regimen was ineffective, however, in blocking the development of an incrementally heightened CNS excitability that eventually progressed into seizures, convulsive fibrillations, etc. Only when the dose of atropine was increased to 16 mg/kg were we able to prevent the development of seizures. Parenthetically, these findings are in agreement with those of McDonough and co-workers (1989), which showed that only high atropine doses were effective in blocking soman-induced seizures and convulsions after exposure to a lethal dose of soman. In addition to seizures, cholinergic symptoms of varying severity such as prostration, ataxia, uncontrolled mucoid/salivary secretions, lacrimation, defecation, urination, hindlimb dystonia, muscular fasciculations, bradycardia, bradypnea and electrocardiographic anomalies (e.g., QT-prolongation, arrhythmias, J-Point elevation, T-inversion) could also be seen 20-60 min following 2 mg/kg atropine treatment. Even when the dose of atropine was raised to 8 mg/kg, some or all of the cholinergic hyperfunctions mentioned above were still observable in 50% of the animals. In the present study, we were able to successfully prevent the development and progression of seizures with scopolamine at a dose as low as 0.1 mg/kg. In addition to the striking anticonvulsant response, animals receiving scopolamine treatment displayed only mild to modest degree of cholinergic hyperfunctions.

The phenomenon of a transient elevation in cortical excitability exhibited by 33% of Group 1 animals (0.1 mg/kg scopolamine) is worthy of further note. The latency (8-12 min post-VR) and power spectral attributes (2-3 times the amplitude of control) of this augmented cortical excitability were very similar to the ECoG activity profile typically seen preceding the development of full-blown seizures in OP-intoxicated animals. The development of this aberrant ECoG pattern suggests that 0.1 mg/kg dose level was probably a bit too low to consistently block the development of a progressively heightened CNS excitability.

Another notable difference in the therapeutic outcome of scopolamine and atropine was the extent to which central respiratory drive was restored. In general, animals receiving either scopolamine or atropine (Chang *et al.*, 2002) all showed a period of compensatory increase in diaphragmatic activities. Power spectral analyses of diaphragmatic EMG data (Fig. 5) showed that, irrespective of the scopolamine dose levels, the diaphragmatic amplitude was consistently returned to a level comparable to that of control 2-3 h following treatment. Examination of diaphragmatic power spectrograms 24 h later also did not reveal any aberrant change in the respiratory functional dynamics and the magnitude of central respiratory drive. In animals treated with atropine (2, 8 or 16 mg/kg), however, the initial compensatory increase in diaphragmatic activities was invariably followed by a sustained period (>6 h) of depression. Further scrutiny of these records revealed that the spectral power reduction in atropine-treated animals appeared to span the entire diaphragmatic frequency spectrum (1-1000 Hz) which suggested a reduction in central respiratory drive. Incidentally, an infirmity of peripheral respiratory mechanism, such as phrenic-diaphragm neuromuscular interface, would have been implicated had the reduction in diaphragmatic spectral amplitudes been limited to any particular spectral ranges or discrete spectral components. These findings suggested that scopolamine could provide a considerably greater degree of protection to the central respiratory mechanism than atropine.

Scopolamine has long been known to possess antidotal action against OP poisoning (e.g., Wescoe *et al.*, 1948; Wills, 1963). Notwithstanding, the use of scopolamine in medical management of OP toxicities pales in comparison to atropine despite evidence that seemed to suggest that scopolamine may be a more effective antidote than atropine (e.g., Anderson *et al.*, 1994; 1997; Bertram *et al.*, 1977; Capacio and Shih, 1991; Harris *et al.*, 1991; 1994; Lennox *et al.*, 1992; Janowsky *et al.*, 1984; Jovic and Milosevic, 1970; Leadbeater *et al.*, 1985; McDonough and Shih, 1993; Solana *et al.*, 1991; Wescoe *et al.*, 1948; Wills, 1963). In addition to its general antidotal effects, research from this institute (Anderson, 1994; 1997; Harris *et al.*, 1994) has shown that scopolamine appeared to be a notably more powerful anticonvulsant than benzodiazepine derivatives (such as diazepam and midazolam) in animals intoxicated by soman (an extremely toxic chemical warfare agent). In consideration of its profound anticonvulsant and anticholinergic properties, these investigators further suggested that scopolamine could possibly replace atropine or diazepam, or both, as a therapeutic compound against soman toxicities. Our findings are very much in agreement with this contention.

That scopolamine can mediate a more effective protection of CNS than atropine is not particularly surprising. Irrespective of the widespread impression that scopolamine and atropine are virtually identical in their pharmacological actions, they do differ qualitatively and quantitatively in their antimuscarinic properties (for review, see Brimblecombe, 1974). In clinical applications, atropine appears to mediate a much less CNS depressant effect in doses that are used medically and is therefore given in preference over scopolamine for most anticholinergic/vagolytic purposes. In applications such as induction of pre-anesthesia where CNS depression is desired or clinically inconsequential, scopolamine is often chosen over atropine. These phenomena are in good agreement with *in vitro* data that showed that, compared with atropine, scopolamine's antimuscarinic activity was about 16-fold more potent in the CNS and about 3-fold more potent in the peripheral neural tissues (Freedman *et al.*, 1988). Thus, to the extent that cholinergic mechanisms are known to play a significant role in OP-induced seizures (Shih *et al.*, 1991) and dysfunctional central respiratory drive (Brimblecombe, 1977), a more centrally active

antimuscarinic compound, such as scopolamine, would appear to have a distinct therapeutic advantage over a less centrally active compound such as atropine.

The exact therapeutic role of scopolamine in VR poisoning awaits further research. The undesirable side effects of scopolamine should also be more clearly defined. Capacio and co-workers (1992) showed that animals did not exhibit performance deficit in an accelerating rotarod test when given an anticonvulsant dose of scopolamine (0.43 mg/kg), which, incidentally, was comparable to the highest dose level (0.5 mg/kg) used in this study. This finding is encouraging since behavioral and electrophysiological correlates derived from the present study showed that a dose of scopolamine as low as 0.1 mg/kg was sufficient to protect the animals from VR-induced central/peripheral perturbations and lethality. Our data also suggest that a considerably greater degree of protection could be expected when scopolamine's dose level was increased to 0.25 or 0.5 mg/kg. Notwithstanding, in view of scopolamine's profound central effect, more systematic research efforts are needed to establish a dose level/range that can provide maximal therapeutic benefit with minimal iatrogenic effect.

In conclusion, while scopolamine and atropine can both prevent the onset of a variety of VR-induced peripheral cholinergic symptoms, our findings showed that scopolamine was far more effective than atropine in inhibiting the development of VR-induced hyperfunctions, seizures and in restoring the functional integrity of central respiratory rhythmogenic mechanism.

REFERENCES

- Anderson, D.R., Gennings, C., Carter, W.H., Harris, L.W., Lennox, W.J., Bowersox, S.L., and Solana, R.P. (1994). Efficacy comparison of scopolamine and diazepam against soman-induced debilitation in guinea pigs. *Fund. Appl. Toxicol.* **22**, 588-593.
- Anderson, D.R., Harris, L.W., Chang, F.-C.T., Baze, W.B., Capacio, B.R., Byers, S.L., and Lennox, W.J. (1997). Antagonism of soman-induced convulsions by midazolam, diazepam and scopolamine. *Drug Chem. Toxicol.* **20**, 115-131.
- Baze, W.B. (1993). Soman-induced morphological changes: an overview in the non-human primate. *J. Appl. Toxicol.* **13**, 173-177.
- Bertram, U., Kasten, A., Lullmann, H., and Ziegler, A. (1977). Improved treatment of organophosphate intoxication by use of scopolamine or dextetimide. *Experientia* **33**, 1196.
- Brimblecombe, R.W. (1974). *Drugs actions cholinergic systems*. University Park Press, Baltimore.
- Brimblecombe, R.W. (1977). Drugs acting on central cholinergic mechanisms and affecting respiration. *Pharmacol. Ther.* **3**, 5-74.
- Capacio, B.R., and Shih, T.-M. (1991). Anticonvulsant actions of anticholinergic drugs in soman poisoning. *Epilepsia* **32**, 604-615.
- Capacio, B.R., Harris, L.W., Anderson, D.R., Lennox, W.J., Gales, V., and Dawson, J.S. (1992). Use of the accelerating rotarod for assessment of motor performance decrement induced by potential anticonvulsant compounds in nerve agent poisoning. *Drug Chem. Toxicol.* **15**, 177-201.
- Chang, F.-C.T., Gouty, S.C., Eder, L.C., Hoffman, B.E., Maxwell, D.M., and K.M. Brecht. (1998). Cardiorespiratory effects of O-isobutyl S-[2-(diethylamino)ethyl]methylphosphonothioate - A structural isomer of VX. *J. Appl. Toxicol.* **18**, 337-347.
- Chang, F.-C.T., Hoffman, B., and DeBus, S. (2002). Pharmacological Antagonism of Lethal Effects Induced by O-isobutyl S-[2-(diethylamino)ethyl]methylphosphonothioate. *Drug Chem. Toxicol.* In Press.
- Chang, F.-C. T., and Harper, R.M. (1989). A procedure for chronic recording of diaphragmatic electromyographic activity. *Brain Res. Bull.* **22**, 561-563.
- Childers, D.G. (1978). *Modern Spectrum Analysis*. IEEE Press, New York.
- Churchill, L., Pazdernik, T.L., Jackson, J.L., Nelson, S.R., Samson, F.E., McDonough, J.H., and McLeod, C.G. (1985). Soman-induced brain lesions demonstrated by muscarinic receptor autoradiography. *Neurotoxicol.* **6**, 81-90.

- Clement, J.G., and Broxup, B. (1993). Efficacy of diazepam and avizafone against soman-induced neuropathology in brain of rats. *Neurotoxicol.* **14**, 485-504.
- Dirnhuber, P., French, M.C., Green, D.M., Leadbeater, L., and Stratton, J.A. (1979). The protection of primates against soman poisoning by pretreatment with pyridostigmine. *J. Pharm. Pharmacol.* **31**, 295-299.
- Dunn, M.A., and Sidell, F.R. (1989). Progress in medical defense against nerve agents. *J. Amer. Med. Assoc.* **262**, 649-652.
- Freedman, S.B., Beer, M.S., and Harley, E.A. (1988). Muscarinic M1, M2 receptor binding: relationship with functional efficacy. *Eur. J. Pharmacol.* **156**, 133.
- Harris, L.W., Talbot, B.G., Lennox, W.J., Anderson, D.R., and Solana, R.P. (1991). Physostigmine (alone and together with adjunct) pretreatment against soman, sarin, tabun and VX intoxication. *Drug Chem. Toxicol.* **14**, 265-281.
- Harris, L.W., Gennings, C., Carter, W.H., Anderson, D.R., Lennox, W.J., Bowersox, S.L., and Solana, R.P. (1994). Efficacy comparison of scopolamine (SCP) and diazepam (DZ) against soman-induced lethality in guinea pigs. *Drug Chem. Toxicol.* **17**, 35-54.
- Hayward, I.J., Wall, H.G., Jaax, N.K., Wade, J.V., Marlow, D.D., and Nold, J.B. (1990). Decreased brain pathology in organophosphate-exposed rhesus monkeys following benzodiazepine therapy. *J. Neurol. Sci.* **98**, 99-106.
- Janowsky, D., Drennan, M., Berkowitz, A., Turken, A., and Risch, S.C. (1984). Anticholinergic antagonism of cholinesterase inhibitor lethality. *Lancet* **ii**, 74.
- Jovic, R., and Milosevic, M. (1970). Effective doses of some cholinolytics in the treatment of anticholinesterase poisoning. *Eur. J. Pharmacol.* **12**, 85-93.
- Keeler, J.R., Hurst, C.G., and Dunn, M.A. (1991). Pyridostigmine used as a nerve agent pretreatment under wartime conditions. *J. Amer. Med. Assoc.* **266**, 693-695.
- Lallement, G., Carpentier, P., Pernot-Marino, L., Baubichon, D., Collet, A., and Blanchet, G. (1991). Involvement of the different rat hippocampal glutamatergic receptors in development of seizures induced by soman: an autoradiographic study. *Neurotoxicol.* **12**, 655-664.
- Lallement, G., Delamanche, I.S., Pernot-Marino, I., Baubichon, D., Denoyer, M., Carpentier, P., and Blanchet, G. (1993). Neuroprotective activity of glutamate receptor antagonists against soman-induced hippocampal damage: quantification with an w3 site ligand. *Brain Res.* **618**, 227-237.
- Lallement, G., Pernot-Marino, I., Baubichon, D., Burckhart, M.-F., Carpentier, P., and Blanchet, G. (1994). Modulation of soman-induced neuropathology with an anticonvulsant regimen. *NeuroRep.* **5**, 2265-2268.

- Leadbeater, L., Inns, R.H., and Rylands, J.M. (1985). Treatment of poisoning by soman. *Fund. Appl. Toxicol.* **5**, S225-S231.
- Lemercier, G., Carpentier, P., Sentenac-Roumanou, H., and Moralis, P. (1983). Histological and histochemical changes in the central nervous system of the rat poisoned by an irreversible anticholinesterase organophosphorus compound. *Acta Neuropathol. (Berl)*. **61**, 123-129.
- Lennox, W.J., Harris, R.L., Anderson, D.R., Solana, R.P., Murrow, M.L., and Wade, J.V. (1992). Successful pretreatment/therapy of soman, sarin and VX intoxication. *Drug Chem. Toxicol.* **15**, 271-283.
- Martin, L.J., Doebler, J.A., Shih, T.-M., and Anthony, A. (1985). Protective effect of diazepam pretreatment on soman-induced brain lesion formation. *Brain Res.* **325**, 287-289.
- Maxwell, D.M., Brecht, K.M., and Koplovitz, I. (1997). Characterization and treatment of the toxicity of O-isobutyl S-[2-(diethylamino)ethyl]methylphosphonothioate, a structural isomer of VX, in guinea pigs. *J. Am. Coll. Toxicol.* **15**(Suppl. 2), S78-S88.
- McDonough, J.H., Jaax, N.K., Crowley, R.A., Mays, M.Z., and Modrow, H.E. (1989). Atropine and/or diazepam therapy protects against soman-induced neural and cardiac pathology. *Fundam. Appl. Toxicol.* **13**, 256-276.
- McDonough, J.H., and Shih, T.-M. (1993). Pharmacological modulation of soman-induced seizures. *Neurosci. Biobehav. Rev.* **17**, 203-215.
- McDonough, J.H., Dochterman, L.W., Smith, C.D., and Shih, T.-M. (1995). Protection against nerve agent-induced neuropathology, but not cardiac pathology, is associated with the anticonvulsant action of drug treatment. *Neurotoxicol.* **15**, 123-132.
- McLeod, C.G. (1985). Pathology of nerve agents: Perspectives on medical management. *Fundam. Appl. Toxicol.* **5**, S10-S16.
- Petras, J.M. (1994). Neurology and neuropathology of soman-induced brain injury: an overview. *J. Exp. Anal. Behav.* **61**, 319-329.
- Shih, T.-M., and McDonough, J.H. (2000). Efficacy of biperiden and atropine as anticonvulsant treatment for organophosphate nerve agent intoxication. *Arch. Toxicol.* **74**, 165-172.
- Shih, T.M., Koviak, T., and Capacio, B. (1991). Anticonvulsants for poisoning by the organophosphorus compound soman: Pharmacological mechanisms. *Neurosci. Biobehav. Rev.* **15**, 349-362.
- Solana, R.P., Gennings, C., Carter, W.H., Anderson, D.R., Lennox, W.J., Carchman, R.A., and Harris, R.L. (1991). Efficacy comparison of two cholinolytics, scopolamine and azapropfen, when used in conjunction with physostigmine and pyridostigmine for protection against organophosphate exposure. *J. Am. Coll. Toxicol.* **10**, 215-222.

Szafraniec, L.L., Beaudry, W.T., and Szafraniec, L.J. (1995). Decontamination chemistry of Russian VX. Proceedings of the Scientific Conference on Chemical and Biological Defense Research. November 14-17, 1995. Aberdeen Proving Ground, Maryland.

Wescoe, W.C., Green, R.E., McNamara, B.P., and Krop, S. (1948). The influence of atropine and scopolamine in the central effects of DFP. *J. Pharmacol. Exp. Ther.* **92**, 63-72.

Wills, J.H. (1963). Pharmacological antagonists of the anticholinesterase agents. In *Cholinesterases and Anticholinesterase Agents*. (Koelle, G.B. ed), Handbuch der Experimentellen Pharmakologie. pp. 883-920, Springer-Verlag, Berlin.