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HEALTH EFFECTS OF PROJECT SHAD CHEMICAL AGENT:

VX NERVE AGENT

[CAS Nos. 50782-69-9, 51848-47-6] [53800-40-1, 70938-84-0]

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SPECIAL NOTE ON PSYCHOGENIC SEQUELAE OF PERCEIVED EXPOSURE TO BIOCHEMICAL WARFARE AGENTS

This report deals primarily with the biological health challenges engendered by the agent that is the subject of the report. Nevertheless, this report also incorporates, by reference and attachment, a supplement entitled "Psychogenic Effects of Perceived Exposure to Biochemical Warfare Agents".

The supplement addresses and describes a growing body of health effects research and interest centered upon the psychogenic sequelae of the stress experienced personally from actual or perceived exposure to chemical and biological weaponry. Because awareness of exposure to agents in Project SHAD logically includes the exposed person also possessing a perception of exposure to biochemical warfare agents, the psychogenic health consequences of perceived exposure may be regarded as additional health effects arising from the exposure to Project SHAD agents. This reasoning may also apply to simulants and tracers. Therefore, a general supplement has been created and submitted under this contract to address possible psychogenic effects of perceived exposure to biological and chemical weaponry.

Because such health effects are part of a recent and growing public concern, it is expected that the supplement may be revised and expanded over the course of this contract to reflect the actively evolving literature and interest in the issue.

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I. EXECUTIVE SUMMARY

VX Nerve Agent (VX) is a chemical warfare nerve agent. Its chemical formula is $C_{11}H_{26}NO_2PS$. Its formal chemical name is O-Ethyl S-(2-diisopropylaminoethyl) methylphosphonothiolate. Due to the existence of several isomers, VX has several CAS (Chemical Abstracts Service) registry numbers: 50782-69-9, 51848-47-6, 53800-40-1, and 70938-84-0.

VX is an organophosphate compound and it belongs to the specific class of compounds known as the phosphonothiocholines. The "V" in VX stands for "venom," a tribute to this compound class having high potency and a characteristic ability to penetrate the skin. At normal temperatures, it is an oily liquid of low volatility with viscosity similar to motor oil.

Ranaji Ghosh first synthesized VX in the early 1950s. The British government noted VX's potential as a warfare agent and shared its research with the U.S Army Edgewood facility. Eventually large quantities of VX were produced through the 1960s at a Newport Indiana facility. Some stocks still remain there and on other bases and have been slated for destruction in 2004. The Soviet Union developed a related compound called Russian VX (O-Isobutyl S-(2-diethylamino) methylphosphonothioate).

VX has been the subject of accidental releases, controlled releases, and has been used as a weapon. The largest reported accidental release occurred at Utah's Dugway Proving Grounds on March 13, 1968 when approximately 9 kg of VX drifted over adjacent grazing land, killing over 6000 sheep. There was also an accidental release of a nerve agent (sources conflict on whether VX was involved) at a storage facility in Okinawa in 1969, which resulted in the hospitalization of 23 military personnel and one civilian. In Project SHAD at least two test releases on ships have been reported.

In addition to releases by the US Army, VX was used by the Aum Shinrikyo cult in Japan to kill several dissident members, and others opposed to the cult. It may have also been used by Iraq as part of a cocktail in the Iran-Iraq war and to quell Kurdish uprisings in the 1980's. US troops were exposed nerve agents during destruction and disposal operations in the Gulf War, though VX is not reported to be among those agents.

VX is a potent and selective inhibitor of acetylcholinesterases (AChE), which results in the accumulation of acetylcholine in the synapses of both central and peripheral nerves. VX, in contrast to other nerve agents inhibits AChE significantly more than plasma cholinesterases. VX exposure and action results in intense stimulation of nicotinic, muscarinic, and central nervous system (CNS) receptors. Toxic effects are generally seen when over 50% of the AChE enzyme is inhibited. Death typically occurs when over 90% of the AChE enzyme is inhibited. Death is usually due to inhibition of the enzyme in the brain and diaphragm.

The increased amounts of acetylcholine in the brain produced by VX exposure leads to the release of large amounts of excitory amino-acids which stimulate NMDA receptors and result in neuronal toxicity. Seizures typically occur when 25%-75% of AChE is inhibited and always occur during exposure to supralethal doses. Convulsions without treatment can lead to permanent neurological damage.

In addition to the inhibition of acetylcholinesterase, VX has been shown to bind to and block postjunctional glutamate receptors, nicotinic acetylcholine receptor-ion channels, and muscarinic acetylcholine receptors. The role of receptor binding and inhibition in toxicity is not clear. Studies in mice in which the acetylcholinesterase gene has been knocked out indicate that other targets of organophosphates may play a major role in toxicity and lethality.

The toxic effects of VX can be grouped around the types of nerve receptors overstimulated by acetylcholine. The muscarinic effects are typically miosis, headaches, blurring of vision, rhinorrhea, bradycardia, anorexia, nausea, vomiting, diarrhea, increased sweating, and lacrimation. The nicotinic effects are typically fatigue, muscular twitching, cramps, and paralysis of muscles (including respiratory muscles). The acute CNS effects are typically generalized weakness, cyanosis, hypotension, convulsions, loss of consciousness, coma, and death. Longer-term CNS effects including anxiety, insomnia, tremor, headaches, drowsiness, difficulty in concentration, memory problems, confusion, slurred speech, and ataxia have been associated with organophosphate poisoning but not VX specifically.

There currently are no commercial test kits that diagnose VX exposure. Diagnosis is from signs and symptoms. Gas chromatography coupled with mass spectrometry (GC-MS) can detect metabolites of VX in both urine and serum. Several tests have been developed that attempt to identify nerve agent poisoning through the quantification of cholinesterase activity in blood. The monitoring of AChE activity is a reliable marker for systemic toxicity. Systemic toxic effects are seen in approximately 50% of subjects when 75% of red blood cell AChE is inhibited. A more recent test relies on the ability of potassium fluoride to reactivate enzymes such as butyryl-cholinesterase and release fluorinated compounds. This technique can be used to monitor low levels of exposures and unambiguously identify both nerve agents and pesticides.

VX is considered to be the most toxic of the nerve agents developed for chemical warfare. Course, symptoms, and relative toxicity, however, can vary considerably by exposure route and dose. The human dermal LD_{50} (Lethal Dose) is estimated to be as low as 0.04 mg/kg; human inhalation LCt_{50} (Lethal Concentration) is estimated to be 36 mg · min/m^3. By inhalation, it is twice as lethal as sarin. It is also ten times more toxic in inducing miosis. VX is at least 100 times more toxic than sarin as a percutaneous agent due to its low volatility, its stability and its lipophilicity.

The effects of exposure by inhalation usually occur within minutes. Miosis, rhinorrhea, and airway constriction are initially seen at low to moderate concentrations. Larger doses of VX result in loss of consciousness, seizures, cessation of cardiac and respiratory

activity and death in the absence of medical treatment. Neuropsychiatric effects including loss of memory and depression are also seen but are relatively short-lived following exposure to VX .

The onset of symptoms can take hours when sublethal doses are applied to the skin. A small drop may initially cause localized muscle twitching and sweating, followed by nausea, vomiting, diarrhea and generalized weakness. These symptoms typically last for several hours. Systematic dermal studies in humans showed vomiting occurred in 33% and 67% of subjects when red blood cholinesterase activity was 30-39% and less than 20% of control activity**.** Other studies have shown that a dose of 5 mg/kg of VX resulted in systemic toxicity in roughly half of the subjects. Persons whose skin is exposed to higher doses of VX may show no symptoms for up to 30 minutes, but then rapidly suffer loss of consciousness, convulsions, difficulty breathing, profuse secretions from nose and mouth, generalized muscle twitching, paralysis and death. At lethal and near-lethal levels of exposure loss of consciousness, convulsions, flaccid paralysis, and apnea are seen. At high doses there is also a more rapid onset of signs and symptoms. Clothing, site of skin exposure, and temperature can greatly affect the nature and toxicity of dermal exposure.

Animal studies have indicated VX can cause cardiac effects, although these effects have not been seen in human volunteer studies. Arrhythmias were seen both in rats and dogs at doses that did not result in convulsions. Electrophysiological studies using guinea pig heart tissue showed that VX exposure led to a positive inotropic effect, two contractile events in response to each stimulation and the development of delayed after-depolarizations**.** VX cardiac toxicity has been attributed to the inhibition of the rat cardiac Na+,K(+)-ATPase alpha 1 isoform.

Few studies have addressed long-term toxicity or effects of nerve agents in general and VX in particular. Textbooks indicate that most if not all of the effects of nerve agents dissipate within months after exposure. The results of a recent telephone survey of over 4000 volunteers who had participated in programs that involved exposure to chemical agents between 1955 and 1975 at Edgewood MD found fewer attention problems as the only statistically significant differences between those exposed to nerve agents and those exposed to other chemical agents but it also found greater sleep disturbances in volunteers who had been exposed to nerve agents. VX differs from other nerve agents in that it does not appear to undergo aging or stabilization but does to undergo spontaneous reactivation

Unlike many other organophosphates, VX also has not been shown to induce a syndrome called organophosphorus-induced delayed neuropathy (OPIDN). OPIDN results from the inhibition of the enzyme neuropathy target esterase (NTE; also termed neurotoxic esterase). VX has been reported to be at least 1000 times less effective than sarin in inhibiting NTE. The failure of VX to inhibit neuropathy target esterase and cause organophosphorus-induced delayed neuropathy together with the inability of to “age” when bound to AchE or other proteins indicates that VX may not cause much of the long-term toxicity associated with other organophosphates.

VX has tested negative in a number of assays for mutagenicity, with and without metabolic activation. Human studies in personnel working with VX on a daily basis found no increased incidence of cancer. The teratogenic potential of VX has also been evaluated in sheep, rats and rabbits; all have all been negative for teratogenicity. VX has not been deemed a carcinogen by any authority.

In regard to long-term neurotoxicity, VX has not been shown to have delayed or persistent psychological effects or to result in any long-term EEG changes. Organophosphorus-induced delayed neuropathy (OPIDN) has not resulted from VX exposure. Convulsions without treatment can lead to permanent neuropathogical damage.

Brain damage has been seen in animals injected with VX. Microinjections of VX into the amygdala resulted in convulsions and resultant neuropathology. Much of the brain damage that has been observed is believed to be due from the induction of convulsions and not the direct toxic actions of VX. Studies on neuroblastoma cells have indicated that VX displays some toxicity presumably through binding to muscarinic receptors.

No studies have been found addressing purely psychogenic effects arising from an awareness of, or a perception of, exposure to VX specifically. But the use of another organophosphate agent (sarin) in terror attacks in Japan in the 1990s has led to some investigation and consideration of the possible psychogenic effects of exposure to a nerve agent. Discussion of those reports appear in the review prepared under this contract for the health effects of sarin. Information on the general psychogenic effects of perceived exposure to biological or chemical warfare agents is contained in the supplement report under this contract "Psychogenic Effects of Perceived Exposure to Biochemical Warfare Agents."

There have been several approaches towards the treatment of, and protection against, VX exposure. Barrier methods, including garments, respirators and even protective creams have been developed that will protect against even high levels of VX exposure. The use of reversible inhibitors of AChE to protect against subsequent exposure to nerve agents has been pursued extensively by the US military. Pyridostigmine bromide was used by a large number of troops during the Gulf War to protect against possible exposure to Soman and other nerve agents. Several studies since then have implicated pyridostigmine as a potential contributory factor in the induction of Gulf War Syndrome, a multi-symptom illness found in a number of veterans who served in Iraq. Other reports have since questioned its utility in protecting against VX exposure.

Several other agents have also been proposed for prophylaxis against nerve agent exposure. Both physostigmine and hyoscine has been reported superior to pyridostigmine in preventing the death of animals following VX exposure. Huperzine has also been found to be a more effective prophylactic agent than pyridostigmine. In contrast to other prophylactic agents, huperzine does not inhibit butyryl-cholinesterase (plasma) which can then still act to scavenge nerve agents.

To prevent mortality and minimize morbidity, aggressive medical intervention should be pursued following nerve agent exposure. Thorough decontamination should occur immediately following suspected exposure. Casualties should be decontaminated as fast as possible but should not be moved into clean treatment areas until decontamination is complete. Bleach should be used extensively to decontaminate any area or material where exposure has occurred. Atropine sulfate, an anticholinergic agent, should be administered as soon as possible following decontamination. Oxygen or oxygen rich air should be used for ventilation if available. Oximes, such as pralidoxime salts, should also be administered as soon as possible to regenerate AChE enzymes. Early intervention to prevent or treat convulsions is also an essential component in the treatment of nerve agent poisoning. Imidazenil, a partial selective allosteric modulator of GABA action, has been shown to be more effective than diazepam in protecting rats against organophosphate-induced convulsions and death.

Secondary literature on VX generally adequately covers its well-known lethality and toxicity. Researchers ought to be cautioned to note that VX, due to varied isomers, has multiple CAS registry numbers.

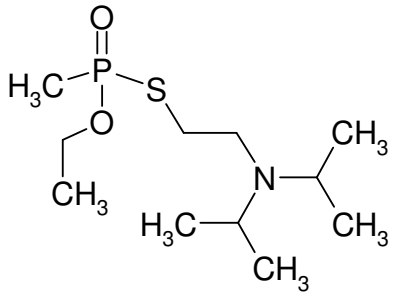
II. BACKGROUND DATA: CHEMISTRY & HISTORY

Chemistry

Sources for this subsection (unless otherwise specified): National Research Council 1999, Mitretek 2004.

Formula: $C_{11}H_{26}NO_2PS$

Structure (display may take a few moments in electronic version of this report):



Names: VX, EA 1701, Edgewood Arsenal No. 1701, T 2445, TX-60
O-Ethyl S-(2-diisopropylaminoethyl) methylphosphonothiolate, Ethyl S-2-diisopropylaminoethyl methylphosphonothiolate, Phosphonothioic acid, methyl-, S-(2-(bis(1-methylethyl)amino)ethyl) O-ethyl ester, (+-)-

CAS Registry Numbers: 50782-69-9, 51848-47-6, 53800-40-1, 70938-84-0

Physical Properties

Molecular Weight: 267.38
Melting Point: -39 ^{o}C (usually listed at less than due to-51^{o} C due to impurities (CBWinfo 2004))
Boiling Point: 298 ^{o}C
Vapor pressure at 20 ^{o}C: 0.0007 mm Hg
Vapor Density: 9.2 (Air = 1)
Density: 1.008 g/mL
Aqueous Solubility: Miscible below 9 ^{o}C; 3 g per 100g at 25 ^{o}C ;5 g per g at 25 ^{o}C

Estimated log Kow: 2.06

VX is an oily viscous liquid substance at normal temperature. Its principal use is as a chemical warfare nerve agent. It is an organophosphate compound, as are nerve agents sarin and soman.

Historical Background

The use of chemical warfare agents is probably nearly as old as war itself. Early documented uses of chemical warfare agents include: the Chinese use of arsenical smoke as early 1000 BC; the use of hellebore roots by Athenian soldiers to poison the drinking water of Kirrha in 600 BC; the use of sulfur dioxide and other noxious fumes by the Spartans against the Athenians during the Peloponnesian Wars; and the use of mandrake root laced wine by the Carthaginians to sedate Roman soldiers in 200 BC. The most extensive use occurred during World War I when the use of both chlorine and mustard gases resulted in over one-million casualties (Eckert 1991, Landersman 2003, Smart 1997).

VX is a member of a more recent class of organophosphate chemical compounds termed phosphorylthiocholines or phosphonylthiocholines. This class was initially discovered independently by Ranaji Ghosh of Imperial Chemicals Inc. (ICI), Gerhard Schrader of Bayer, and by Lars-Erik Tammelin of the Swedish Institute of Defense Research in 1952-1953. The compounds were first developed as insecticides, but soon thereafter the human toxicity of the compounds also became evident. One of the compounds, Amiton, (VG; O,O-Diethyl-S-[2-(diethylamino)ethyl] phosphorothioate) was actually brought to market by ICI but withdrawn after discovery of its human toxicity. By 1953, the British recognized the potential of these compounds as chemical warfare agents. (The V in the V-class of phosphorylthiocholine compounds stands for "venom", a term derived from the V-class members' high potency and ability to penetrate the skin (CBWInfo 2004, Mitretek 2004, Reutter 1999, Smart 1997)).

First synthesized by Ghosh around 1955, VX is the most widely studied of the phosphorylthiocholine class of compounds. After its synthesis, the toxicity and physical properties of VX were studied by chemical warfare specialists at Porton Down in Britain, who shared their information with the US Army's Edgewood facility. Those investigations led to VX being chosen as the US Army's second-generation nerve agent. A patent dispute, requiring at one point an injunction from the Chief Justice of the United States for its resolution, delayed the onset of production. Finally, in 1959, the FMC Corporation was awarded a contract for the mass production of VX. A former Atomic Energy plant in Newport, Indiana, which produced heavy water, was adapted by FMC for VX production. The plant was designed to make up to 10 tons of VX per day. It operated from 1961 to 1968 (Smart 1997). The Army also developed a number of shells, rocket missiles and even land mines that were capable of dispersing VX. Many of these weapons were stored at Army bases throughout the world (Smart 1997).

There are still over 1200 tons of VX stored at the Newport facility. The destruction of the stockpile has been scheduled to begin during the summer of 2004. Destruction is set to be carried out by hydrolyzing VX with water and sodium hydroxide at a temperature of 195^{o}C (CNN 2004).

VX has been involved in several accidental releases and several controlled releases by the US military. The largest reported accidental release occurred at Utah's Dugway Proving Grounds on March 13, 1968 when a portion or all of over 9 kg of sprayed VX drifted over adjacent grazing land, killing over 6000 sheep. There was also an accidental release of a nerve agent at a storage facility in Okinawa in 1969, which resulted in the hospitalization of 23 military personnel and one civilian. One source has reported that this incident involved VX (CBWInfo 2004), while another source states the incident involved sarin (Smart 1997).

Controlled releases of VX reported by the Army as part of Project SHAD include: Flower Drum Phase 2 in 1964, when VX was sprayed onto a barge and "Fearless Johnny" in 1965 which the George Eastman, a Navy cargo ship, was also sprayed. Both of the releases were meant to test decontamination and protective equipment and both occurred off the coast of Hawaii (Special Assistant 2004).

The accidental releases coupled with reports of intentional releases led to action by Congress and President Nixon to ban the production of biological weapons and to severely limit the development and production of chemical weapons. Although Nixon effectively ended the production of US chemical weapons, research continued on binary weapons. Binary weapons are weapons in which two less toxic chemicals, initially separated in a shell, are mixed upon firing and undergo a chemical reaction to form a potent chemical warfare agent. The Army ultimately did develop a binary weapon, code named Bigeye, which was capable of forming VX upon firing. Bigeye, however, never went into full production. The collapse of the Soviet bloc spelled the end of the Army's offensive chemical warfare program. On June 1, 1990, the United States and the Soviet Union signed a bilateral agreement to destroy all chemical weapons. This effectively ended the US offensive chemical weapons program (Smart 1997).

Although the Soviet Union did not develop VX, they developed a very similar compound usually called Russian VX (R-VX; O-Isobutyl S-(2-diethylamino) methylphosphonothioate). The production of this chemical did lead to slow chronic and apparently irreversible toxicity in workers who produced the nerve agent (Gur'eva et al. 1997).

In addition to releases by the US Army, VX was developed and used by the Aum Shinrikyo in Japan to kill several dissident members and others thought to be opposed to the cult. It may have also been used by Iraq as part of a cocktail in the Iran-Iraq war or to quell Kurdish uprisings in the 1980's (CBWInfo 2004). The following subsection details these actual and reported uses of VX as a weapon.

Use As Terror or Military Weapon

Aum Shinrikyo, a religious cult founded in Japan by Asahara Shoko in the 1980's, used both VX and sarin to kill opponents and create panic throughout Japan. Although the sarin attacks on a residential area in Matsumoto in 1994 and on the subway in Tokyo in 1995 are well known, the cult experimented and used a number of other toxic substances

including VX. VX was reportedly used to kill as many as twenty people including dissident cult members, opponents of the cult, and members of the general population. Aum Shinrikyo also used VX in several attacks that were unsuccessful. Most victims were directly squirted with VX in the head or neck region, although in one case that was unsuccessful, VX was applied to the door handle of a car that the intended victim used (Monterey Institute, 2001, Tsuchihashi et al. 1998).

The synthesis of both VX and sarin by the cult appears to have been accomplished in a relatively short period of time by a group of chemists from Japanese universities under the supervision of Masami Tsuchiya, a former Ph.D. student at Tsukuba University. Mr. Tsuchiya was recently sentenced death for his role in the synthesis of these toxins (BBC 2004). The ability of Aum Shinrikyo to synthesize small quantities of VX from commercially available starting materials in a non-industrial setting indicates that VX may be used in the future by both government and non-government sponsored organizations (Department of Defense 1998).

Iraq, under Saddam Hussein, was reported to have produced over 50 tons of VX. VX may have been used as part of a cocktail in attacks against the Kurds and against Iranians during the Iran-Iraq war. There also is evidence that Iraq weaponized VX and deployed it in artillery shells. There is no evidence, however, that Saddam Hussein intentionally used VX or any other nerve agents during the Gulf War, although there is evidence that low levels of nerve agents were released as result of the bombing of munitions dumps and the destruction of munitions by coalition troops. One of these incidents did lead to exposure of US troops to nerve agents when rockets were destroyed at the Khamisiyah Pit. Those nerve agents, however, appear to have been sarin and cyclosain, not VX (CIA 2002).

There have also been reports that Al Qaeda pursued VX, although it is unlikely that they were successful. One of the justifications for the bombing of a pharmaceutical plant in Sudan was that it was producing VX. This was later shown to be untrue. It is doubtful that Al Qaeda currently has the technical capability to manufacture VX (Council on Foreign Relations 2004, CBWInfo. 2004).

III. PATHOPHYSIOLOGY & DIAGNOSIS

VX is a potent and selective inhibitor of acetylcholinesterases (AChE) and its toxicity is largely a consequence of irreversible inhibition of AChE, which results in the accumulation of acetylcholine in the synapses of both central and peripheral nerves. VX, in contrast to other nerve agents, suppresses the activity of AChE significantly more than plasma cholinesterases (Sidell et al. 1974).

VX's action results in intense stimulation of nicotinic, muscarinic, and central nervous system (CNS) receptors. Toxic effects are generally seen when over 50% of the AChE enzyme is inhibited. Death typically occurs when over 90% of the AChE enzyme is inhibited (Moretto 1998). Death is usually due to inhibition of the enzyme in the brain and diaphragm. The overstimulation of nicotinic, muscarinic, and CNS receptors produces distinct effects for each type of receptor. (These are listed in detail in the section IV on Health Effects.).

The increased amounts of acetylcholine in the brain produced by VX exposure stimulate seizure activity that leads to the release of large amounts of excitory amino-acids which stimulate NMDA receptors and result in neuronal toxicity. Seizures typically occur when 25%-75% of AchE is inhibited; they nearly always occur at exposure to supralethal doses. Convulsions without treatment can lead to permanent neuropathogical damage (Tuovinen 2004).

In addition to the inhibition of acetylcholinesterase, VX has been shown to bind to and block postjunctional glutamate receptors (Albuquerque 1985), nicotinic acetylcholine receptor-ion channels (Rao et al. 1987), and muscarinic acetylcholine receptors (Bakry et al. 1988). The role of receptor binding and inhibition in toxicity is not clear. Studies in mice in which the acetylcholinesterase gene has been knocked out indicate that other targets of organophosphates may play a major role in toxicity and lethality (Duysen et al. 2001).

Diagnosis

There are currently no commercial available kits that confirm VX or nerve agent exposure. Diagnosis is therefore usually based on signs and symptoms. As noted in the next section, these can vary substantially based on route and dose.

Although there are no routine tests, several reports have shown that utilizing gas chromatography coupled with mass spectrometry (GC-MS) can detect metabolites of VX in both urine and serum (Tsuchihasi et al. 1998, Miki et al. 1999, Driskell et al. 2002, Hayes et al. 2004). One report identified the metabolites, 2-(Diisopropylaminoethyl)methyl sulfide (DAEMS), Ethyl methylphosphonic acid (EMPA) in the serum of a VX poisoning victim. DAEMS appears to be formed by the methylation of 2-(Diisopropylamino)ethanethiol (DAET) mediated by a thiol methyltransferase. Both DAET and EMPA are both formed during the spontaneous or

enzymatic hydrolysis of VX. In the particular case studied, VX poisoning was not confirmed until six months after the victim's death (Tsuchihasi et al. 1998).

In addition to specifically identifying VX or nerve agent metabolites, several tests have been developed that attempt identify nerve agent poisoning through the quantification of cholinesterase activity in blood. The monitoring of AChE activity in blood has been shown to be a reliable marker for systemic toxicity. There are two types of cholinesterase (ChE) in blood: red blood cell ChE, which is very similar to ChE released at nerve synapses, and a nonspecific plasma ChE (butyryl cholinesterase or pseudocholinesterase). Red blood cell AChE is very similar to AChE released at nerve synapses. Systemic toxic effects are seen in approximately 50% of subjects when 75% of their red blood cell AChE is inhibited (Munro et al. 1994).

The Test-Mate OP Kit developed by the EQM Corporation has been chosen by the Army to monitor potential organophosphate poisoning. The test kit is compact and uses a single drop of blood to measure levels of both acetylcholinesterase present in red blood cells and butyryl-cholinesterase or pseudocholinesterase. The test is to be used in the field and in clinical labs to monitor levels of organophosphate exposure (Garcia et al. 2004).

A more recent test relies on the ability of potassium fluoride to reactivate plasma enzymes such as butyryl-cholinesterase. Upon reactivation a phosphofluoridate or phosphonofluoridate is formed which can then be identified and quantitated using gas chromatography. This technique is very sensitive and can be used to monitor low levels of exposures. It can also be used retrospectively to determine if exposure occurred weeks or even months previously. This technique can unambiguously identify both nerve agents and pesticides (Van Der Schans et al. 2004).

IV. HEALTH EFFECTS

Overview

VX is considered to be the most toxic of the nerve agents developed for chemical warfare. When the route of administration is inhalation, it is twice as lethal as sarin and ten times more toxic in inducing miosis. As a percutaneous agent, VX is at least 100 times more toxic than sarin due to its low volatility, its stability and its lipophilicity (Reutter 1999).

In the absence of medical treatment, large doses of VX result in loss of consciousness, seizures, cessation of cardiac and respiratory activity and death. The effects of exposure by inhalation usually occur within minutes with miosis, rhinorrhea, and airway constriction initially seen at low or moderate concentrations. The onset of symptoms can take hours when sublethal doses are applied to the skin.

Vomiting and nausea are the usual initial effects of VX, followed by muscular weakness. Neuropsychiatric effects including loss of memory and depression are also seen but are relatively short-lived following exposure to VX (Sidell et al. 1997). The type and extent of effects are dependent on both the dose and route of exposure.

As noted in the previous section, the overstimulation of muscarinic, nicotinic, and CNS receptors by organophosphate agents result in distinct sets of possible symptoms related to the type of receptor.

Muscarinic effects are typically the following:

Miosis
Headaches
Blurring of vision
Rhinorrhea
Bradycardia
Anorexia
Nausea
Vomiting
Diarrhea
Increased sweating
Lacrimation.

Nicotinic effects are typically the following:

Fatigue
Muscular twitching
Cramps
Paralysis of muscles, including respiratory muscles.

CNS effects are typically the following:

Acute:

Generalized weakness
Cyanosis
Hypotension
Convulsions
Loss of consciousness
Coma
Death

Chronic/Long-term (noted in organophosphate poisoning generally but not specifically reported for VX):

Anxiety
Insomnia
Tremor
Headaches
Drowsiness
Difficulty in concentration
Memory problems
Confusion
Slurred speech
Ataxia

(Gunderson et al. 1992).

Acute Toxicity Data

Acute toxicity data for VX has also been compiled in a variety of species; the lethal dose (LD) and lethal concentration (LCt) levels are listed below (Somani et al. 2001).

Species	Route	LD_{50} ; LCt_{50}	Units
Human	Inhalation	36	$mg \cdot min/m^3$
Monkey	Inhalation	50	$mg \cdot min/m^3$
Dog	Inhalation	15	$mg \cdot min/m^3$
Rabbit	Inhalation	25	$mg \cdot min/m^3$
Guinea Pig	Inhalation	8-30	$mg \cdot min/m^3$
Rat	Inhalation	17	$mg \cdot min/m^3$
Mice	Inhalation	7-40	$mg \cdot min/m^3$
Human	Dermal	0.04-0.14	mg/kg
Monkey	Dermal	0.065	mg/kg
Pig	Dermal	0.4	mg/kg
Dog	Dermal	0.054	mg/kg

Cat	Dermal	0.012	mg/kg
Rabbit	Dermal	0.025	mg/kg
Rat	Dermal	0.1	mg/kg
Mice	Dermal	0.046	mg/kg
Human	Intravenous	8	µg/kg
Goat	Intravenous	5	µg/kg
Dog	Intravenous	6.3	µg/kg
Cat	Intravenous	2.5	µg/kg
Rabbit	Intravenous	8.4	µg/kg
Guinea Pig	Intravenous	4.5	µg/kg
Rat	Intravenous	7.9	µg/kg
Mice	Intravenous	14.1	µg/kg
Rat	Subcutaneous	21	µg/kg
Hen	Intramuscular	30	µg/kg

US Army Exposure Guidelines

The US Army has proposed acute emergency guideline levels (AEGLs) for VX. The table below describes these levels.

Guideline Level	10-min Conc.	30-min Conc.	1-hour Conc,	4-hour Conc,	8-hour Conc.
AEGL-1 nondisabling	0.0002 mg/m^3	0.00011 mg/m^3	0.00008 mg/m^3	0.00004 mg/m^3	0.000028 mg/m^3
AEGL-2 disabling	0.0024 mg/m^3	0.0014 mg/m^3	0.00098 mg/m^3	0.00049 mg/m^3	0.00035 mg/m^3
AEGL-3 lethal	0.0096 mg/m^3	0.0049 mg/m^3	0.0033 mg/m^3	0.0017 mg/m^3	0.0013 mg/m^3

Since actual animal and human inhalation studies were lacking for VX, these levels were derived from the relative potency of VX compared to sarin (Hartmann 2002).

Additional Acute Neurological Effects

A wide variety of acute behavior effects have been seen in a number of subjects exposed to VX. These include fatigue, jitteriness or tenseness, inability to read with comprehension, feeling of being mentally slowed, depression, irritability, listlessness, poor performance on serial 7s and other simple arithmetic tests, minor difficulties in orientation (Sidell 1997).

Dermal Exposure

VX has the viscosity of motor oil but it is less volatile. It is significantly more toxic than any other nerve agent following skin exposure. Unlike G-type agents, VX can act predominantly via the percutaneous route. VX is not detoxified by the skin and reacts very little, if at all, with plasma cholinesterases (Munro et al. 1994).

The amount of VX absorbed is dependent on the thickness and penetrability of skin as well the temperature. Studies on guinea pigs and marmosets indicate that approximately 2.5% of VX applied to the skin reaches the blood. These studies also indicated that isomers of VX have equivalent bioavailability (Van der Schans et al. 2003). Studies in swine showed that bioavailability varied depending on the site of application. VX applied in the ear region showed much greater bioavailability than that applied to the epigastrium, perineum, or inguinal crease (Duncan et al. 2002).

Studies in human volunteers have also shown that VX absorption is highly dependent on site and temperature during exposure. The fraction of the applied dose of VX that penetrated the skin when applied to the cheek ranged from 3.5% at –18 ^{o}C to 31.9% at 46 ^{o}C over a three hour time period. Only 0.4% at –18 ^{o}C to 2.9% at 46 ^{o}C penetrated the skin when VX was applied to the forearm (Craig et al. 1977). The time between VX exposure and the maximal effect can also depend on the site of exposure. In studies where small equipotent amounts VX were applied to the skin, the maximal effect was seen at 5 hours when applied to the head or neck, 7 hours when applied to the extremities, and 10 hours when applied to the torso (Sidell 1997).

Clothing can also have an effect upon VX absorption. Dry clothing has been estimated to reduce the dermal toxicity of VX 6- to10-fold. Sweated clothing, however, will only reduce adsorption by 3-fold (Munro et al. 1994, Wester et al. 2000).

Signs and symptoms following exposure to the skin begin within 0.5-18 hours. A small drop may at first cause localized muscle twitching and sweating, followed by nausea, vomiting, diarrhea and generalized weakness. These symptoms typically last for several hours. Systematic dermal studies in humans showed vomiting occurred in 33% and 67% of subjects when red blood cholinesterase activity was 30-39% and less than 20% of control activity. Other studies have shown that a dose of 5 µg/kg of VX resulted in systemic toxicity in roughly half of the subjects (National Research Council (US) 1999).

Persons whose skin is exposed to higher doses of VX may show no symptoms for up to 30 minutes, but thereafter may rapidly suffer loss of consciousness, convulsions, difficulty breathing, profuse secretions from nose and mouth, generalized muscle twitching, paralysis and death. At lethal and near-lethal levels of exposure loss of consciousness, convulsions, flaccid paralysis, and apnea are seen. At high doses there is also a more rapid onset of signs and symptoms (Sidell 1997, Emedicine 2004).

Oral Exposure

The low volatility and environmental persistence of VX make ingestion a relevant potential route of human exposure. Studies involving human volunteers have shown that that the oral toxicity is about 3-fold less than IV toxicity. At oral doses ranging from 0.002-0.0045 mg/kg only a few subjects (5/32) displayed gastrointestinal signs or symptoms. No changes were observed in the heart rate, blood pressure, mental performance, and pupil size in any of the subjects. Eating prior to ingestion also appeared to increase the bioavailability of VX (Sidell et al. 1974). An earlier study also showed no effect in volunteers who were orally dosed at 0.00143 mg/kg over a period of 7 days (Munro et al. 1994).

Intravenous (IV) Exposure

Several studies have addressed the IV toxicity of VX in humans. A group that received 0.0015 mg/kg saw a significant decrease in mental performance for one hour after the injection. Nausea, vomiting were seen as well (Sidell et al. 1974). Subjects who received 0.001 mg/kg over a 1.75- to 4-hr period showed no signs of toxicity, other than a headache in one patient, even though there was a 50-60% reduction in red blood cell AChE. Studies in which 0.00008 mg/kg was administered over a 30 sec period resulted in headaches, lightheadedness and abdominal cramps, even though there was no decrease in red blood cell AChE (National Research Council (US) 1999).

Inhalation Exposure

Signs and symptoms from vapor exposure generally manifest within seconds or minutes. These typically include miosis or pinpoint pupils, eye pain, running nose, difficulty breathing and productive coughing (Emedicine 2004). Nausea and vomiting may also occur, but the onset is usually later. Exposure to higher doses results in severe dyspnea, along with gastrointestinal and neuromuscular effects. Exposure to lethal or near lethal doses leads to loss of consciousness, flaccid paralysis, convulsions and apnea (Sidell 1997).

Cardiac Effects

Although cardiac effects were not seen in any of the studies involving human volunteers, animal studies have indicated VX may cause cardiac effects. Arrhythmias were seen both in rats and dogs at doses that did not result in convulsions (Robineau 1987, Robineau et al. 1987). Electrophysiological studies using guinea pig heart tissue showed that VX exposure led to a positive inotropic effect, two contractile events in response to each stimulation and the development of delayed after-depolarizations (Corbier et al. 1989**).** VX cardiac toxicity in rats has been attributed to the inhibition of the rat cardiac Na+,K(+)-ATPase alpha 1 isoform. At 1 μM concentration VX inhibited the cardiac Na+,K(+)-ATPase by 35%. This inhibition is believed to account for cardiac toxicity seen with VX (Robineau et al. 1991).

Long-Term Effects: General

There have been few studies that have addressed long-term toxicity or effects of nerve agents in general and VX in particular. Textbooks have indicated that most if not all of the effects of nerve agents dissipate within months after exposure (Sidell 1997). The results of a recent telephone survey of over 4000 volunteers who had participated in programs that involved exposure to chemical agents between 1955 and 1975 at Edgewood MD did not indicate any major health problems associated with exposure to nerve agents. The only statistically significant differences between those exposed to nerve agents and those exposed to other chemical agents were fewer attention problems and greater sleep disturbances in volunteers exposed to nerve agents (Page 2003).

VX differs from other nerve agents in that it does not appear to undergo aging or stabilization but does to undergo spontaneous reactivation. The rate of reactivation of red blood cell AChE appears to be about 1% per hour (Sidell et al. 1974).

Unlike many other organophosphates, VX also has not been shown to induce a syndrome called organophosphorus-induced delayed neuropathy (OPIDN). OPIDN results from the inhibition of the enzyme neuropathy target esterase (NTE; also termed neurotoxic esterase). VX has been reported to be at least 1000 times less effective than sarin in inhibiting NTE (Gordon et al. 1983, Vranken et al. 1982). The failure of VX to inhibit neuropathy target esterase and cause organophosphorus-induced delayed neuropathy together with the inability of to "age" when bound to AchE or other proteins indicates that VX may not cause much of the long-term toxicity associated with other organophosphates.

Long-Term Effects: Mutagenicity/Carcinogenicity/Teratogenicity

VX has tested negative in a number of assays for mutagenicity, with and without metabolic activation. These studies included the Ames test (bacteria), yeast, fruit-flies, and mammalian cells (mouse lymphoma cells). VX also gave negative results in a sister chromatid exchange (SCE) assay. Human studies in personnel working with VX on a daily basis found no increased incidence of cancer. The teratogenic potential of VX has also been evaluated in a number of species. Tests in sheep, rats and rabbits have all been negative for teratogenicity. (Munro et al. 1994, National Research Council (US) 1999).

Long-Term Effects: Neurotoxicity

VX has not been shown to have delayed or persistent psychological effects or to result in any long term EEG changes. (Sidell 1997). Also, as noted above, organophosphorus-induced delayed neuropathy (OPIDN) has not resulted from VX exposure. Certain long-term chronic effects have been reported from organophosphate poisoning generally. These have included anxiety, insomnia, tremor, headaches, drowsiness, difficulty in concentration, memory problems, confusion, slurred speech, ataxia (Gunderson et al.

1992). Again, it is not clear whether human exposure specifically to VX will result in long term effects since VX inhibited enzymes and receptors undergo spontaneous reactivation and do not appear to undergo aging (Sidell et al. 1974). No studies report or ascribe long-term human neurological clinical health effects to VX exposure.

Brain damage has been seen in animals injected with VX. Microinjections of VX into the amygdala resulted in convulsions and resultant neuropathology. Injection of 3.4 nanomoles of VX into the amygdala of rats resulted in convulsions and brain damage in 67% of the animals treated. It should be noted that much of the brain damage that has been observed is believed secondary in nature to VX exposure, as it is regarded to be due to the induction of convulsions and not to the results of direct toxic actions by VX (McDonough et al. 1987). Convulsions induced by organophosphate agents, without subsequent treatment, have been shown to lead to permanent neuropathological damage (Tuovinen 2004).

In vitro studies on human neuroblastoma cells have indicated that VX displays some direct cytotoxicity presumably through binding to muscarinic receptors (Cao et al. 1999).

V. PSYCHOGENIC EFFECTS

No studies have been found addressing psychogenic effects arising from an awareness of, or a perception of, exposure specifically to VX. But the use of another organophosphate agent (sarin) in terror attacks in Japan in the 1990s led to some investigation and consideration of the possible psychogenic effects of exposure to a nerve agent. Discussion of those reports appear in the review prepared under this contract for the health effects of sarin.

Information on the general psychogenic issues and effects of perceived exposure to biological or chemical warfare agents is contained in the supplement report under this contract "Psychogenic Effects of Perceived Exposure to Biochemical Warfare Agents."

VI. TREATMENT & PREVENTION

There have been several approaches for the treatment of, and protection against VX exposure. Barrier methods, including garments, respirators and even protective creams have been developed that will protect against even high levels of VX exposure. Vapor protective composite garments consisting of non-woven materials bonded to vapor resistant film or garments with activated charcoal embedded in them are effective against VX. Self-contained breathing apparatuses (SCBA) and air-purifying respirators both protect against VX exposure (Jirka 2001). Israeli researchers have developed a protective cream that was shown to be effective against both VX and mustard gas exposures (Kadar et al. 2003).

The use of reversible inhibitors of AChE to protect against subsequent exposure to nerve agents has been pursued extensively by the US military. Pyridostigmine bromide was used by a large number of troops during the Gulf War to protect against possible exposure to Soman and other nerve agents. Several studies have implicated pyridostigmine as a potential contributory factor in the induction of Gulf War Syndrome, a multi-symptom illness found in a number of veterans who served in Iraq. A comprehensive report by the Rand Corporation indicated that current data is unable to conclusively prove or rule out a role for pyridostigmine in Gulf War Syndrome. This report also questioned the effectiveness of pyridostigmine in preventing nerve agent toxicity (Golumb et al. 1999). Several animal studies indicate that pyridostigmine pretreatment interferes with subsequent post-exposure therapy using oximes and atropine. In comparison to other nerve agents, the reduction in efficacy was pronounced in animals following VX exposure (Koplovitz et al. 1992).

Several other agents have also been proposed for prophylaxis against nerve agent exposure. Both physostigmine and hyoscine has been reported superior to pyridostigmine in preventing the death of animals following VX exposure (Wetherell et al. 2002). Huperzine, a compound currently under investigation for the treatment of dementia, was also a more effective prophylactic agent than pyridostigmine. In contrast to other prophylactic agents, huperzine does not inhibit butyryl-cholinesterase (plasma). Butyryl-cholinesterase can then still act to scavenge nerve agents, and as a result, huperizine is believed to be the most effective agent for preventing organophosphate poisoning (Lallement et al. 2002).

To prevent mortality and minimize morbidity, aggressive medical intervention should be pursued following nerve agent exposure. Thorough decontamination should occur immediately following suspected exposure. The subject should be removed from the site of exposure; all clothing should be removed. Any potential droplets of nerve agent should be blotted, and not wiped, away. Absorbent powders such as flour can also be used for removal.

A subject's skin and hair should be extensively washed with large amounts of soap and water but water and soap from the hair should not come in contact with the skin. A decontamination solution consisting of either household bleach or 10% sodium bicarbonate can also be used. Casualties should be decontaminated as fast as possible but should not be moved into clean treatment areas until decontamination is complete. Bleach should be used extensively to decontaminate any area or material where exposure has occurred (CBWInfo. 2004).

Atropine sulfate, an anticholinergic agent, should be administered to the exposed individual as soon as possible following decontamination. An initial intravenous dose of 2 mg should be given, followed by additional doses every 10-15 minutes until bradycardia subsides. Intramuscular injections should be considered if the patient is hypoxic and ventilation cannot be initiated. Oxygen or oxygen rich air should be used for ventilation if available. Oximes, such as pralidoxime salts, should also be administered as soon as possible to help regenerate AChE enzymes. Typically, 500 mg–1 g of pralidoxime is administered through a slow intravenous infusion (CBWInfo. 2004, Newmark 2004). Several other oximes such has K048, HI-6, obidoxime have been shown to reactivate AChE enzymes. K048 has been reported to be the more effective than the other agents in reactivating AChE inhibited by VX (Kuca et al. 2003). Another oxime, TO205, has recently been reported to be the most efficacious agent the reactivation of VX-inhibited AChE (Kuca et al. 2004).

Early intervention to prevent or treat convulsions is an essential component in the treatment of nerve agent poisoning. An initial 5 mg dose of diazepam, a full allosteric modulator of GABA action, followed by additional doses every 15 minutes up to 15 mg is typically given following VX exposure (CBWInfo. 2004). Imidazenil, a partial selective allosteric modulator of GABA action, has been shown to be more effective than diazepam in protecting rats against organophosphate-induced convulsions and death (Auta et al. 2004).

VII. SECONDARY SOURCE COMMENT

Literature on VX generally adequately covers its lethality and toxicity. Researchers are cautioned to note that VX, due to varied isomers, has multiple CAS registry numbers.

VIII. BIBLIOGRAPHY WITH ABSTRACTS

{Unless otherwise noted, the abstracts for the following references are rendered verbatim as provided by the original publication or as made available in a standard print or electronic catalogue or database. Errors, omissions, or other defects of style or substance are strictly those of the original source.}

Albuquerque et al. 1985. Multiple actions of anticholinesterase agents on chemosensitive synapses: molecular basis for prophylaxis and treatment of organophosphate poisoning. *Fundam Appl Toxicol*. 5(6 Pt 2):S182-203.
The present study demonstrates that the reversible and irreversible anti-ChE agents have direct actions on the nicotinic acetylcholine receptor-ionic channel (AChR) and on the locust glutamatergic neuromuscular junction. In addition, the prophylaxis of lethality of organophosphorus anti-ChE compounds was studied. The lethality of VX and sarin was diminished when the rats were pretreated with physostigmine and atropine. The effectiveness of this protection, however, was markedly increased when a ganglionic blocker, either mecamylamine or chlorisondamine, was added, such that all the animals survived after receiving four times a lethal dose of VX. Pretreated animals receiving sarin showed significant recovery of morphological and functional properties of the neuromuscular junction as compared to the damage of structures from animals without pretreatment. Blood ChE inhibition was slightly decreased while brain and muscle AChE levels were significantly recovered (from 98 and 70% to 56 and 32%, respectively) by the pretreatment. This effect may partially explain the protection given by physostigmine but not that afforded by addition of a non-anti-ChE agent. Physostigmine, at concentrations greater than 20 microM, showed both a marked depression of the peak amplitudes of the endplate current (EPC) and a shortening of the decay time constants tau EPC. These effects were mostly due to a direct drug interaction with the nicotinic AChR blocking the ionic channel in its open conformation. Single-channel recordings showed that physostigmine decreases conductance and open times of the channels activated in the presence of ACh and in addition has an agonistic property on the nicotinic AChR. VX, on the other hand, only shortened the open times of ACh-activated channels without affecting the conductance. No agonist property was detected with VX. On glutamatergic synapses, the ChE inhibitors generated spontaneous firing of end-plate potentials (EPPs) and action potentials (APs). This effect was blocked in the presence of low external Ca2+ concentration or tetrodotoxin. It seems that the spontaneous EPP and AP firing resulted from an increased transmitter release induced by an increase in Na+ influx at the presynpatic nerve terminal. Physostigmine and some irreversible ChE inhibitors (VX and DFP) also blocked the postjunctional glutamate receptors. Similar to the nicotinic AChR, this effect was mostly related to a blockade of the open channels. In conclusion, the present studies showed significant protection of rats by physostigmine in combination with some ganglionic antagonists against lethality by organophosphate agents.

Auta et al. 2004. Imidazenil: a potent and safe protective agent against diisopropyl fluorophosphate toxicity. *Neuropharmacology.* 46(3):397-403.
Convulsions are major and life-threatening signs of organophosphate (OP) nerve agents induced neurotoxicity. Thus, early intervention with anticonvulsant drugs to control seizure propagation and the consequent irreversible neuronal damage that may occur during OP exposure is essential. Diazepam is the standard anticonvulsant used in the therapeutic management of OP poisoning. However, its use has been associated with several unwanted effects including, sedation, amnesia, and in the large doses used for such treatment, respiratory depression. Moreover, protracted administration of diazepam has been associated with tolerance and dependence liabilities. In this study, we compared the efficacy and safety of diazepam (full allosteric modulator of GABA action) to that of imidazenil (partial, selective allosteric modulator of GABA action) as preventive treatment against diisopropyl fluorophosphate (DFP)-induced convulsions and mortality. Our results show that imidazenil is more potent and efficacious than diazepam in protecting rats against DFP-induced convulsions and death. Moreover, imidazenil was effective at doses (1 and 0.5 mg/kg) we have previously shown to be devoid of sedation, amnesia, respiratory depression, or tolerance and/or dependence. In contrast, diazepam was effective at doses (5 and 2.5 mg/kg) that produce sedation, amnesia, and ataxia. Furthermore, the combination of imidazenil with atropine was more potent and efficacious than that with diazepam.

Bakry et al. 1998. Direct actions of organophosphate anticholinesterases on nicotinic and muscarinic acetylcholine receptors. *J Biochem Toxicol*. 3:235-59.
Four nerve agents and one therapeutic organophosphate (OP) anticholinesterase (anti-ChE) bind to acetylcholine (ACh) receptors, inhibit or modulate binding of radioactive ligands to these receptors, and modify events regulated by them. The affinity of nicotinic (n) ACh receptors of Torpedo electric organs and most muscarinic (m) ACh receptors of rat brain and N1E-115 neuroblastoma cultures for the OP compounds was usually two to three orders of magnitude lower than concentrations required to inhibit 50% (IC-50) of ACh-esterase activity. However, a small population of m-ACh receptors had an affinity as high as that of ACh-esterase for the OP compound. This population is identified by its high-affinity [3H]-cis-methyldioxolane ([3H]-CD) binding. Although sarin, soman, and tabun had no effect, (O-ethyl S[2-(diisopropylamino)ethyl)] methyl phosphonothionate (VX) and echothiophate inhibited competitively the binding of [3H]-quinuclidinyl benzilate ([3H]-QNB) and [3H]-pirenzepine ([3H]-PZ) to m-ACh receptors. However, VX was more potent than echothiophate in inhibiting this binding and 50-fold more potent in inhibiting carbamylcholine (carb)-stimulated [3H]-cGMP synthesis in N1E-115 neuroblastoma cells--both acting as m receptor antagonist. All five OPs inhibited [3H]-CD binding, with IC-50s of 3, 10, 40, 100, and 800 nM for VX, soman, sarin, echothiophate, and tabun, respectively. The OP anticholinesterases also bound to allosteric sites on the n-ACh receptor (identified by inhibition of [3H]-phencyclidine binding), but some bound as well to the receptor's recognition site (identified by inhibition of [125I]-alpha-bungarotoxin binding). Soman and echothiophate in micromolar concentrations acted as partial agonists of the n-ACh receptor and induced receptor desensitization. On the other hand, VX acted as an open channel blocker of the activated receptor and also enhanced receptor desensitization. It is suggested that the

toxicity of OP anticholinesterases may include their action on n-ACh as well as m-ACh receptors if their concentrations in circulation rise above micromolar levels. At nanomolar concentrations their toxicity is due mainly to their inhibition of ACh-esterase. However, at these low concentrations, many OP anticholinesterases (eg, VX and soman) may affect a small population of m-ACh receptors, which have a high affinity for CD. Such effects on m-ACh receptors may play an important role in the toxicity of certain OP compounds.

Cao et al. 1999. Cytotoxicity of organophosphate anticholinesterases. *In Vitro Cellular & Developmental Biology Animal* 5 (9):493-50. Organophosphate (OP) anticholinesterases were found to modulate metabolic activities of human neuroblastoma cells and hepatocytes, which was detectable by the Cytosensor microphysiometer. The nerve gas ethyl-S-2-diisopropylaminoethyl methylphosphorothiolate (VX), at 10 muM, produced significant reduction in cell metabolism within 2 min, as measured by changes in the acidification rate of the medium. The reduction was dose- and time-dependent and irreversible after 4 h of exposure. Two alkaline opropylfluorophosphate or chlorpyrifos gave an LC50 of 65, 775, 640, 340, or 672 muM, respectively, whereas 24 h gave an LC50 of 0.7, 3.7, 2.5, 29, and 31 muM, respectively. Preincubation of hepatocytes with phenobarbital enhanced their response to parathion and VX due to metabolic bioactivation. Atropine partially blocked the effects of VX and paraoxon on both cell types, which suggests the involvement of a muscarinic receptor as the target for cytotoxicity.

CIA 2002. Intelligence Update: Chemical Warfare Agent Issues During the Persian Gulf War. April 2002 . Available at http://www.cia.gov/cia/publications/gulfwar/cwagents/cwpaper1.htm After conducting a multiyear study, we assess that CW agents reached US Persian Gulf war troops in only one case—the 10 March 1991 inadvertent release of nerve agent from the US demolition of Iraqi chemical rockets in a pit at the Khamisiyah Depot in Iraq. That release resulted in low-level nerve agent contamination of a significant area as published in the joint DoD-IC paper *Modeling the Chemical Warfare Agent Release at the Khamisiyah Pit*, 4 September 1997. In 2000, DoD published revised modeling of the Khamisiyah Pit release using updated CIA source assessments; this new modeling indicates that the area used for troop notification of potential low-level exposure has decreased by roughly half compared to the results published in 1997. However, the number of US troops to which DoD sent letters of notification actually increased slightly because of better information about their locations.

CBWInfo. 2004. Nerve Agent: VX. Available at http://www.cbwinfo.com/Chemical/Nerve/VX.html - 0008.

CNN 2004. Army to destroy deadly nerve gas 'Most lethal chemical on the planet.' *CNN* June 10, 2004.

Corbier et al. 1989. Evidence for a direct noncholinergic effect of an organophosphorous compound on guinea-pig papillary muscles: are ventricular

arrhythmias related to a Na+/K+ ATPase inhibition? *Arch Int Pharmacodyn Ther* 300:218-30.
The purpose of this study was to determine whether an anticholinesterase organophosphorous compound, methylphosphonothiolate (VX), could display direct effects on isolated guinea-pig ventricular muscle, and to compare these possible effects with those of carbachol (CCH) and physostigmine (PHYS). Our results confirm the direct positive inotropic effect of CCH (stimulation frequency; 2 Hz) in a concentration range from 10(-7) to 10(-3) M; lack of PHYS or VX-induced modifications was set. In the presence of an adrenergic agonist, isoproterenol (ISO) 10(-7) M, CCH or PHYS modulated the positive inotropic effect of ISO. Even with elevated concentrations of CCH (10(-4) M) or PHYS (10(-3) M), arrhythmias were never depicted. VX-induced modifications are different. Under VX, the development of (1) a positive inotropic effect and (2) two contractile events in response to each stimulation were observed. Electrophysiological studies revealed that VX led to the development of delayed after-depolarizations, and eventually triggered activity. We conclude that, in addition to its anticholinesterase activity, VX could induce a Na+/K+ ATPase inhibition. This effect could be at the onset of ventricular arrhythmias that are observed in vivo in organophosphorous compounds poisoning.

Council on Foreign Relations 2004. VX: Questions and Answers. Available at http://cfrterrorism.org/weapons/vx.html.

Department of Defense 1998. The Militarily Critical Technologies List Part II: Weapons of Mass Destruction Technologies. *Section 4. Chemical Weapons Technology.* Available at http://www.fas.org/irp/threat/mctl98-2/p2sec04.pdf .
Discusses the various synthetic processes that can lead to the production of VX and other chemical warfare agents.

Driskell et al. 2002. Quantitation of organophosphorus nerve agent metabolites in human urine using isotope dilution gas chromatography-tandem mass spectrometry. *J Anal Toxicol.* 26(1):6-10.
An isotope dilution gas chromatography-tandem mass spectrometric (GC-MS-MS) method was developed for quantitating the urinary metabolites of the organophosphorus nerve agents sarin, soman, tabun (GA), VX, and GF. Urine samples were concentrated by codistillation with acetonitrile, derivatized by methylation with diazomethane, and analyzed by GC-MS-MS. The limits of detection were less than 4 microg/L for all the analytes except for the GA metabolite, which had a limit of detection of less than 20 microg/L.

Duncan et al. 2002. Site-specific percutaneous absorption of methyl salicylate and VX in domestic swine. *J Appl Toxicol.* 22(3):141-8.
The site specificity of the percutaneous absorption of methyl salicylate (MeS) and the organophosphate nerve agent VX (O-ethyl S-(2-diisopropylaminoethyl) methylphosphonothioate) was examined in anaesthetized domestic swine that were fully instrumented for physiological endpoints. Four different anatomical sites (ear, perineum, inguinal crease and epigastrium) were exposed to the MeS and the serum levels were

measured over a 6-h time period. The dose absorbed at the ear region was 11 microg cm(-2) with an initial flux of 0.063 microg cm(-2)min(-1), whereas at the epigastrium region the dose absorbed was 3 microg cm(-2) with an initial flux of 0.025 microg cm(-2)min(-1). For this reason further studies were carried out with VX on the ear and the epigastrium only. In animals treated with agent on the epigastrium, blood cholinesterase (ChE) activity began to drop 90 min after application and continued to decline at a constant rate for the remainder of the experiment to ca. 25% of awake control activity. At this time there were negligible signs of poisoning and the medical prognosis was judged to be good. In contrast, the ChE activity in animals receiving VX on the ear decreased to 25% of awake control values within 45 min and levelled out at 5-6% by 120 min. Clinical signs of VX poisoning paralleled the ChE inhibition, progressing in severity over the duration of the exposure. It was judged that these animals would not survive. The dramatic site dependence of agent absorption leading to vastly different toxicological endpoints demonstrated in this model system has important ramifications for chemical protective suit development, threat assessment, medical countermeasures and contamination control protocols.

Duysen et al. 2001. Evidence for Nonacetylcholinesterase Targets of Organophosphorus Nerve Agent: Supersensitivity of Acetylcholinesterase Knockout Mouse to VX Lethality. *J Pharmacol Exp Ther.* 299(2):528-35.
The possibility that organophosphate toxicity is due to inhibition of targets other than acetylcholinesterase (AChE, EC 3.1.1.7) was examined in AChE knockout mice. Mice (34-55 days old) were grouped for this study, after it was determined that AChE, butyrylcholinesterase (BChE), and carboxylesterase activities had reached stable values by this age. Mice with 0, 50, or 100% AChE activity were treated subcutaneously with the nerve agent VX. The LD50 for VX was 10 to 12 µg/kg in AChE/, 17 µg/kg in AChE+/, and 24 µg/kg in AChE+/+ mice. The same cholinergic signs of toxicity were present in AChE/ mice as in wild-type mice, even though AChE/ mice have no AChE whose inhibition could lead to cholinergic signs. Wild-type mice, but not AChE/ mice, were protected by pretreatment with atropine. Tissues were extracted from VX-treated and untreated animals and tested for AChE, BChE, and acylpeptide hydrolase activity. VX treatment inhibited 50% of the AChE activity in brain and muscle of AChE+/+ and +/ mice, 50% of the BChE activity in all three AChE genotypes, but did not significantly inhibit acylpeptide hydrolase activity. It was concluded that the toxicity of VX must be attributed to inhibition of nonacetylcholinesterase targets in the AChE/ mouse. Organophosphorus ester toxicity in wild-type mice is probably due to inhibition or binding to several proteins, only one of which is AChE.

Eckert 1991. Mass deaths by gas or chemical poisoning. A historical perspective. *Am J Forensic Med Pathol.* 12(2):119-25.
This review chronicles the characteristics of deliberate and accidental mass poisonings that occurred in World Wars I and II, in Bhopal, and in other historical cases up to and including modern wars. It also considers approaches to the investigation of such cases from the medicolegal as well as general standpoints.

Emedicine 2004. Chemical Warfare: Nerve Agents. Available at http://www.emedicinehealth.com/articles/8733-3.asp.

Garcia et al. 2004. WRAIR protocols for soldier status and readiness to organophosphate exposure: unprocessed whole blood cholinesterase and pyridostigmine bromide quantification. Available at http://www.asc2004.com/23rdASC/summaries/d/DP-14.pdf.

Golumb et al. 1999. A Review of the Scientific Literature As It Pertains to Gulf War Illnesses; Pyridostigmine Bromide. Available at http://www.rand.org/publications/CT/CT164/CT164.pdf

Gordon et al. 1983. The delayed neuropathic effects of nerve agents and some other organophosphorus compounds. *Arch Toxicol* 52:71-82.
The in vitro inhibitory potencies of several nerve agents and other organophosphorus compounds against acetylcholinesterase (AChE) and neurotoxic esterase (NTE) were compared. Although the I50 (median inhibitory concentration) against AChE were ê 0.1-1.0 nM for the nerve agents, the I50 against NTE for sarin, soman and tabun were 2-4 orders of magnitude higher and VX (ethyl S-diisopropylaminoethyl methylphosphonothiolate) had negligible activity. A series of bis(omega-phenyl-n-alkyl)phosphorofluoridates inhibited both enzymes at 1.0-100 nM while omega-phenyl-n-alkyl N,N-dimethylphosphoramidofluoridates were active at 0.1-10 muM. From the in vitro data it was predicted that nerve agents would cause delayed neuropathy only at doses greatly exceeding the LD50. In hens protected against acute toxicity by pretreatment with physostigmine, atropine and the oxime P2S (1-methyl,2-hydroxyiminomethylpyridinium methanesulfonate), delayed neuropathy associated with high inhibition of NTE was found at 30-60D50 for soman or 82ed of the latter 2 compounds the inhibition of NTE was 55% and 66%, respectively. The minimum neuropathic doses were ê 100-150uropathy, associated with a high level of inhibition of NTE, was caused by 1 bis-phenylalkyl phosphorofluoridates at doses causing negligible acute toxicity. The required dose was 9 times that for DFP although the compound was 300 times more active against NTE in vitro suggesting that such compounds were rapidly degraded in vivo. The phenylalkyl N,N-dimethylphosphoramidofluoridates produced prolonged acute signs of poisoning but they were not neuropathic at the maximum tolerable doses nor was the NTE greatly inhibited contrary to the prediction from the in vitro data. The enantiomer responsible for the inhibition of NTE was possibly preferentially degraded in vivo. Several other phosphoramidofluoridates inhibited NTE in vitro at 1.0-100 muM and a number of bicyclic phosphates were inactive at 23 muM. None of these compounds was tested in vivo.

Gunderson et al. 1992. Nerve agents: a review. *Neurology* 42:946-50.
Nerve agents produce neuromuscular blockade and convulsions in exposed humans. Military personnel in areas of potential exposure take prophylactic pyridostigmine. They are instructed to self-administer atropine and pralidoxime at the first sign of nerve agent toxicity. The key to treatment of nerve agent poisoning is the administration of atropine in doses larger than is customary in most other disorders, repeated as often as needed.

Mechanical ventilation may be required. Convulsions are treated with diazepam, but only after atropine has been administered.

Gur'eva et al. 1997. Chronic poisoning by organophosphoric VX. *Med Tr Prom Ekol*. (6):7-11.
Long-term observation of workers engaged into production of VX chemical diagnosed slow progressing manifestation of chronic occupational poisoning with the chemical. The characteristic nervous, digestive, locomotory, visual and cardiovascular symptoms were revealed. The authors presented laboratory and instrumental data on the cases. As the treatment appeared ineffective, further adjustment and improvement of the therapy is required. The article demonstrated the main underlying metabolic disorders that could be addressed by the pathogenetic therapy.

Hartmann 2002. Evaluation of risk assessment guideline levels for the chemical warfare agents mustard, GB, and VX. *Regul Toxicol Pharmacol.* 35(3):347-56.
The U.S. Army has estimated acute lethality guideline levels for inhalation of the chemical warfare agents mustard, GB, and VX. These levels are expressed as dosages measured in milligram-minutes per cubic meter (mg-min/m(3)). The National Advisory Council has also proposed acute emergency guideline levels (AEGLs) for the agents. The AEGLs are threshold exposure limits for the general public for mild effects, serious adverse effects, and lethality. They are expressed as air concentrations (in units of mg/m(3)) and are applicable to emergency exposure periods ranging from 10 min to 8 h. The report discusses strengths and deficiencies in the levels, important parameters (i.e., exposure time, breathing rate) that need to be explicitly addressed in deriving the guideline levels, and possible impacts that could result from using AEGLs instead of guideline dosages in future assessments.

Hayes et al. 2004. Feasibility of direct analysis of saliva and urine for phosphonic acids and thiodiglycol-related species associated with exposure to chemical warfare agents using LC-MS/MS. *J Med Chem Def* 2:1-23 (9 Aug 2004) available at http://jmedchemdef.org/Issue_0201/Kenny_0804.html.
A sensitive method based on high performance liquid chromatography tandem massspectrometry (LC-MS/MS) has shown the feasibility of separation and detection of lowlevelthiodiglycol-related species in saliva and of nerve agent phosphonic acids in salivaand urine. The analysis of these thiodiglycol-related species and phosphonic acids are ofinterest since they are specific metabolites of the chemical warfare agents (CWAs) sulfurmustard (HD), Sarin (GB), Soman (GD), GF, and VX. The liquid chromatographyatmosphericpressure chemical ionization tandem mass spectrometry (LC-APCI-MS/MS)and liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESIMS/MS) methods developed provide a sensitive and direct approach for determiningCWA exposure in non-extracted non-derivitized samples from saliva and urine.Chromatographic separation of the thiodiglycol-related species was achieved using areverse phase high performance liquid chromatography (HPLC) column with an isocraticmobile phase of 93% water, 20 mM formic acid, 20 mM ammonium formate, and 7%methanol. Chromatographic separation of the phosphonic acids was achieved using areverse phase HPLC with gradient mobile phases consisting of 0.1%

acetic acid in waterand 0.1% acetic acid in methanol. Identification and quantification of species wasachieved using both atmospheric pressure chemical ionization and electrosprayionization-tandem mass spectrometry monitoring two precursor-to-product ion transitionsfor each compound, as well as internal standards. The concentration vs response waslinear between 10 ng/mL and 500 ng/mL. Instrument detection limits ranged from 10ng/mL to 50 ng/mL.

Jirka 2001. WMD Protective Clothing for the First Responder. *EMS Magazine* . Available at http://www.emsmagazine.com/newsarticles/protclothing.html.

Kadar et al. 2003. A topical skin protectant against chemical warfare agents. *Isr Med Assoc J.* 5(10):717-9.
BACKGROUND: Sulfur mustard and VX are potent chemical warfare agents that penetrate rapidly through the skin, causing severe prolonged injuries and sometimes death. OBJECTIVES: To develop a topically applied pretreatment that will act as a barrier and prevent the absorption of these agents through the skin, reducing morbidity and saving life. METHODS: Several formulations were developed and tested in preclinical animal studies in pigs. The protecting cream was applied as a single application (0.5-1 ml/100 cm2) prior to exposure (10 minutes to 12 hours) to sulfur mustard or VX. Assessment of sulfur mustard-induced skin damage was based on clinical and histologic evaluations. When tested against VX, clinical signs and blood cholinesterase activity were monitored. At the final stage of development, safety studies were conducted in animals and in human volunteers. RESULTS: The formulation that gave the best results, coded IB1 (under patent application), provided significant protection against a 1 hour exposure to sulfur mustard (droplets or vapor). All the pigs pretreated with IB1 cream survived a 1-4 hour challenge of 2xLD50 VX and did not exhibit any overt clinical signs. Protection was exhibited even when the cream was applied 12 hours (single application) prior to exposure. IB1 was found to be non-irritating in animals and humans. No adverse effects were found in a Phase I clinical study in young healthy volunteers when the cream was applied to around 20% of the skin surface (results presented elsewhere). CONCLUSIONS: IB1 cream has been shown to be a safe and effective topical skin protectant against the chemical warfare agents sulfur mustard and VX.

Koplovitz et al. 1992. Reduction by pyridostigmine pretreatment of the efficacy of atropine and 2-PAM treatment of sarin and VX poisoning in rodents. *Fundam Appl Toxicol.* 18(1):102-6.
This study concerned the effect of pyridostigmine pretreatment on (a) the antidotal efficacy of atropine and 2-PAM in sarin, tabun, and VX poisoning in mice and guinea pigs and on (b) the oxime-induced reactivation of VX-inhibited whole blood acetylcholinesterase (AChE) of guinea pigs. One hour prior to organophosphate (OP) challenge with sarin, tabun, or VX, animals were given oral doses of pyridostigmine to induce approximately 30 and 60% inhibition of whole blood AChE; controls received vehicle. Mice were challenged im and guinea pigs sc with the OP compounds. Treatment with atropine (11.2 mg/kg to mice; 32 mg/kg to guinea pigs) plus 2-PAM (25 mg/kg) was given im at 10 sec postchallenge in mice and 1 min postchallenge in guinea pigs. In the

reactivation experiments, pyridostigmine or saline was given im to guinea pigs 30 min prior to VX (8.24 micrograms/kg, sc), atropine (16 mg/kg) was given im at 1 min, and 2-PAM (25 mg/kg) at 16 min postchallenge. Pyridostigmine significantly enhanced the efficacy of atropine and 2-PAM against tabun in both species. In contrast, pyridostigmine reduced or did not increase the efficacy of atropine and 2-PAM against sarin or VX in both species. Recovery of VX-inhibited AChE by 2-PAM was decreased significantly in pyridostigmine pretreated animals. The results suggest that pyridostigmine pretreatment may adversely effect the efficacy of atropine and 2-PAM as antidotes for VX and sarin intoxication.

Kuca et al. 2003. A comparison of the ability of a new bispyridinium oxime--1-(4-hydroxyiminomethylpyridinium)-4-(4-carbamoylpyridinium)butane dibromide and currently used oximes to reactivate nerve agent-inhibited rat brain acetylcholinesterase by in vitro methods. *J Enzyme Inhib Med Chem.* 18(6):529-35.
The efficacy of a new bispyridinium oxime 1-(4-hydroxyiminomethylpyridinium)-4-(4-carbamoylpyridinium)butane dibromide, called K048, and currently used oximes (pralidoxime, obidoxime, the oxime HI-6) to reactivate acetylcholinesterase inhibited by various nerve agents (sarin, tabun, cyclosarin, VX) was tested by in vitro methods. The new oxime K048 was found to be a more efficacious reactivator of nerve agent-inhibited acetylcholinesterase than pralidoxime (in the case of VX, tabun and cyclosarin), obidoxime (cyclosarin and tabun) and HI-6 (tabun) but it did not reach the efficacy of currently used oximes for the reactivation of acetylcholinesterase inhibited by sarin. Thus, the oxime K048 seems to be a relatively efficacious broad spectrum acetylcholinesterase reactivator and, therefore, it could be useful for the treatment of a nerve agent-exposed population if information about detection of the type of nerve agent is not available.

Kuca et al. 2004. Oximes-induced reactivation of rat brain acetylcholinesterase inhibited by VX agent. *Hum Exp Toxicol.* 23(4):167-71.
A comparison of one mono- and seven bisquaternary acetylcholinesterase (AChE) reactivators of acetylcholinesterase inhibited by VX agent was performed. As a source of the acetylcholinesterase, a rat brain homogenate was taken. There were significant differences in reactivation potency of all tested oximes. The oxime TO205 seems to be the most efficacious followed by TO046, HI-6, HS-6, K027, obidoxime, MMC and 2-PAM. In addition, the results of this study showed that the reactivation potency of the tested reactivators depends on many factors--such as the number of pyridinium rings, the number of oxime groups and their position, as well as the length and the shape of linkage bridge between two pyridinium rings.

Lallement et al. 2002. Review of the value of huperzine as pretreatment of organophosphate poisoning. *Neurotoxicology.* 23(1):1-5.
Today, organophosphate (OP) nerve agents are still considered as potential threats in both military or terrorism situations. OP agents are potent irreversible inhibitors of central and peripheral acetylcholinesterases. Pretreatment of OP poisoning relies on the subchronic administration of the reversible acetylcholinesterase (AChE) inhibitor pyridostigmine (PYR). Since PYR does not penetrate into the brain, it does not afford protection against

seizures and subsequent neuropathology induced by an OP agent such as soman. Comparatively, huperzine (HUP) is a reversible AChE inhibitor that crosses the blood-brain barrier. HUP is presently approved for human use or is in course of clinical trials for the treatment of Alzheimer's disease or myasthenia gravis. HUP is also used as supplementary drug in the USA for correction of memory impairment. Besides, HUP has also been successfully tested for pretreatment of OP poisoning. This review summarizes the therapeutical value of HUP in this field. Moreover, the modes of action of HUP underlying its efficacy against OP agents are described. Efficacy appears mainly related to both the selectivity of HUP for red cell AChE which preserves scavenger capacity of plasma butyrylcholinesterases for OP agents and to the protection conferred by HUP on cerebral AChE. Finally, recent data, showing that HUP seems to be devoid of deleterious effects in healthy subjects, are also presented. Globally, this review reinforces the therapeutical value of HUP for the optimal pretreatment of OP poisoning.

Landersman 2003. Chemical and Biological Warfare: Facts and Trends. *Proceedings (Naval Institute) 2003* Web edition. Available at http://www.usni.org/Proceedings/Articles03/prolandersmaniraq.htm - chemicals

McDonough et al. 1987. Direct microinjection of soman or VX into the amygdala produces repetitive limbic convulsions and neuropathology.
Rats were injected in the amygdala and other forebrain sites with nmolar amounts of the highly toxic organophosphate 'nerve agent' compounds soman or VX (O-ethyl-S-(2-diisopropylaminoethyl)-methylphosphonothioate) in an attempt to determine the mechanism(s) responsible for the permanent brain pathology that has been observed following systemic intoxication with these agents. Injections were performed using a stereotaxically guided microsyringe in animals maintained under halothane/oxygen anesthesia or using chronically implanted cannulae in conscious animals. Bilateral microsyringe injections of up to 11.0 nmol soman into the amygdala failed to evoke abnormal behavior or brain pathology. When rats were pretreated with lithium chloride, or when carbachol was coadministered, soman injections evoked repetitive clonic convulsions and neuropathology. Unilateral injections of 3.4 nmol of VX into the amygdala elicited convulsions and brain damage in 67% of the animals tested. Atropine pretreatment (15.0 mg/kg, i.p.) prevented the development of convulsions and brain damage. Neuropathology was observed only in animals that developed repetitive convulsions; the piriform and entorhinal cortex, amygdala, hippocampus and thalamus were the brain structures most consistently damaged. With unilateral injections, the damage was more severe on the side ipsilateral to the injection. The behavioral topography of the convulsions and the neuroanatomical distribution and nature of the subsequent pathology closely resemble that observed with systemic administration of these compounds. The results indicate that the nerve agents are not directly neurotoxic, that peripherally induced hypoxia or anoxia are unlikely mechanisms of the neuropathology, and that the brain damage produced by these compounds is primarily seizure-mediated.

Miki et al. 1999. Determination of alkylmethylphosphonic acids, the main metabolites of organophosphorus nerve agents, in biofluids by gas chromatography-mass

spectrometry and liquid-liquid-solid-phase-transfer-catalyzed pentafluorobenzylation. *J Anal Toxicol.* 23(2):86-93.
A simple gas chromatography-mass spectrometry (GC-MS) procedure has been developed for the main metabolites of organophosphorus nerve agents, alkylmethylphosphonic acids (AMPAs; alkyl = Et, i-Pr, and pinacolyl) in biofluids via extractive pentafluorobenzylation. The derivatization was carried out under liquid-liquid-solid-phase-transfer conditions using a polymer-bound tri-n-butylmethylphosphonium bromide as a catalyst. AMPAs in aqueous samples were semiquantitatively extracted into a small-volume organic layer as their pentafluorobenzyl derivatives at pH 4.5 (85 degrees C). Sample pretreatments for urine, serum, and saliva were each examined to minimize matrix interference. The detection limits of APMAs by electron-impact ionization GC-MS were around 50 ng/mL and 2.5-10 ng/mL in the full-scan and selected-ion monitoring modes, respectively. In order to detect trace-level AMPAs, negative-ion chemical ionization (NICI) was also employed to enhance sensitivity. The detection limits of AMPAs in biofluids were typically 60 pg/mL by GC-NICI-MS.

Mitretek. 2004. The Chemistry of VX. Available at http://www.mitretek.org/home.nsf/homelandsecurity/VX .

Monterey Institute of International Studies 2001. Chronology of Aum Shinrikyo's CBW Activities. Available at http://cns.miis.edu/pubs/reports/pdfs/aum_chrn.pdf .

Moretto 1998. Experimental and clinical toxicology of anticholinesterase agents. *Toxicol Lett.* 102-103:509-13.
Several organophosphorus compounds (OP) and carbamates (CA) are used as insecticides or warfare agents (OPs only). Their acute toxic effect in the central and peripheral nervous system is due to inhibition of acetylcholinesterase (AChE) at nerve endings which causes accumulation of acetylcholine and consequently overstimulation of the nicotinic and muscarinic receptors. The cholinergic syndrome appears at approximately 50% AChe inhibition whereas death is believed to occur at > 90%. Inhibition of AChE (phosphorylation) by most OPs is irreversible whereas CAs reversibly inhibit AChE (spontaneous reactivation with a t(1/2) of minutes); dimethylphosphorylated AChE partially and slowly (t(1/2) = 1-2 h) reactivates. Although long-term, mild neurobehavioural changes of questionable significance have been reported in some instances, recovery from the cholinergic syndrome appears to be complete, unless lesions develop in the central nervous system as a consequence of either convulsions or anoxia. Certain OPs and CAs have been reported to interact with cholinergic receptors in vitro. The toxicological relevance of these interactions is still not clear. Certain OPs cause OP-induced delayed polyneuropathy (OPIDP) which develops 2-5 weeks after an acute poisoning. The molecular target is believed to be neuropathy target esterase (NTE). OP insecticides are more potent AChE inhibitors rather than NTE inhibitors and therefore, the dose required to cause OPIDP is much higher than that causing the cholinergic syndrome. In the experimental animal, OPIDP is associated with > 70% NTE inhibition after single or repeated exposures. The threshold in man is not known, although there are indications that it is similar. Some non-neuropathic esterase inhibitors (OPs, CAs, sulfonyl fluorides) exacerbate the clinical outcome of OPIDP and other chemical

axonopathies, and of nerve crush. The phenomenon has been called promotion and has so far been observed in experimental animals only.
Brain Res. 1987 Dec 1;435(1-2):123-37

Munro et al. 1994. Toxicity of the organophosphate chemical warfare agents GA, GB, and VX: implications for public protection. *Environ Health Perspect* 102:18-38. Available at http://ehp.niehs.nih.gov/members/1994/102-1/munro-full.html .
The nerve agents, GA, GB, and VX are organophosphorus esters that form a major portion of the total agent volume contained in the U.S. stockpile of unitary chemical munitions. Congress has mandated the destruction of these agents, which is currently slated for completion in 2004. The acute, chronic, and delayed toxicity of these agents is reviewed in this analysis. The largely negative results from studies of genotoxicity, carcinogenicity, developmental, and reproductive toxicity are also presented. Nerve agents show few or delayed effects. At supralethal doses, GB can cause delayed neuropathy in antidote-protected chickens, but there is no evidence that it causes this syndrome in humans at any dose. Agent VX shows no potential for inducing delayed neuropathy in any species. In view of their lack of genotoxcity, the nerve agents are not likely to be carcinogens. The overreaching concern with regard to nerve agent exposure is the extraordinarily high acute toxicity of these substances. Furthermore, acute effects of moderate exposure such as nausea, diarrhea, inability to perform simple mental tasks, and respiratory effects may render the public unable to respond adequately to emergency instructions in the unlikely event of agent release, making early warning and exposure avoidance important. Likewise, exposure or self-contamination of first responders and medical personnel must be avoided. Control limits for exposure via surface contact of drinking water are needed, as are detection methods for low levels in water or foodstuffs.

National Research Council (US) 1999. Subcommittee on Chronic Reference Doses for Selected Chemical-Warfare Agents. Review of the U.S. Army's Health Risk Assessments for Oral Exposure to Six Chemical-Warfare Agents Appendix D, National Academy Press. Available at http://books.nap.edu/books/0309065984/html/197.html - pagetop .

Newmark 2004. Therapy for nerve agent poisoning. *Arch Neurol.* 61(5):649-52.
Neurologists need to familiarize themselves with nerve agents, the most toxic of the chemical warfare agents. Their mode of action lies within the nervous system, and nonneurologists will look to neurologists for expert advice on therapy. These agents cause rapid-onset cholinergic crisis amenable to prompt treatment with specific antidotes. Experience on the battlefield and in terrorist attacks demonstrates that therapy saves lives.

Page 2003. Long-term health effects of exposure to sarin and other anticholinesterase chemical warfare agents. *Mil Med.* 168(3):239-45.
In a telephone survey of 4,022 military volunteers for a 1955-1975 program of experimental exposures to chemical agents at Edgewood, Maryland, the current health of those exposed to anticholinesterase agents was compared with that of men exposed to no active chemicals (no chemical test) and to two or more other types of chemical agents (other chemical tests). The survey posed questions about general health and about

neurological and psychological deficits. There were only two statistically significant differences: volunteers in anticholinesterase agent tests reported fewer attention problems than those in other chemical tests and greater sleep disturbance than those in no chemical tests. In contrast, volunteers who reported exposure to civilian or military chemical agents outside of their participation in the Edgewood program reported many statistically significant adverse neurological and psychological effects, regardless of their experimental exposure. In this study, the health effects of self-reported, nonexperimental exposure, which are subject to recall bias, were greater than the health effects of experimental exposure.

Rao et al. 1987. Noncompetitive blockade of the nicotinic acetylcholine receptor-ion channel complex by an irreversible cholinesterase inhibitor. *J Pharmacol Exp Ther*. 240(1):337-44

Abstract:
Interactions of O-ethyl S-[2-(diisopropylamino)ethyl]methylphosphonothioate (VX), an irreversible, organophosphorus, anticholinesterase (anti-ChE) agent, with the nicotinic acetylcholine receptor-ion channel complex (AChR) of the frog Rana pipiens were investigated using electrophysiological techniques. At low concentrations (0.1-0.5 microM) of VX, typical effects due to cholinesterase (ChE) inhibition, such as potentiation of indirect muscle twitches as well as increases in the peak amplitude and decay time constant (tau EPC) of end-plate currents (EPC), were observed. At concentrations greater than or equal to 1.0 microM, VX produced opposite effects. The indirectly elicited muscle twitches and EPC peak amplitude were depressed with an IC50 of about 33 microM. tau EPC was reduced, and at a higher concentration of 100 microM, VX split the decays into faster and slower components. Similar results were also obtained with the amplitude and decays of miniature end-plate currents (MEPC). However, although the MEPC peak amplitude and tau MEPC were not decreased to levels below control values, EPC peak amplitude (but not its tau EPC) was markedly depressed beyond the control values, in a concentration-dependent manner. The fact that nerve action potential-evoked events were more affected than the spontaneous MEPCs suggested an interference with the depolarization-evoked release process. Indeed, VX caused a reduction of the quantal content and, in addition, induced a significant increase in the frequency of MEPCs. All these effects, except the anti-ChE activity, were reversible upon wash. The results from both EPC fluctuation analysis and single channel recordings disclosed a concentration-dependent shortening of the channel open times without change in single channel conductance.

Reutter et al. 1999. Evaluation of Airborne Exposure Limits for VX: Worker and General Population Exposure Criteria. ECBC-TR-074. Edgewood Chemical and Biological Center, Aberdeen Proving Ground, Md., December 1999 NTIS Order No. ADA 375312.

Existing occupational airborne exposure limits (now referred to as “worker population limits” or WPLs) and general population limits airborne limits (now referred to as “general population limits” or GPLs) were reviewed and recalculated using current risk assessment methods and two sets of data not considered in previous estimates. The “newer” data resulted in estimated WPLs and GPLs lower than existing values. However

the quality of both sets of data was such that there was not sufficient confidence to select either as a critical study. Overall the quality and quantity of data for VX are less than desirable. In addition no chronic studies have been done. Given this, it was decided to develop the exposure limits relative to GB. A potency ratio of 10 was selected, based upon relative potencies of GB and VX in producing miosis. (It was noted, however, that unlike GB, VX vapor is a percutaneous hazard, and the relative potency ratio for percutaneous effects is about 100.) Based upon the miosis potency ratio, the existing WPL for VX (0.00001 mg/m3) was deemed adequately protective. However, the existing GPL was not, and a concentration of 0.0000003 mg/m3 is recommended. The existing immediately dangerous to life or health (IDLH) exposure level for occupational workers was recalculated relative to that for GB. A value of 0.01 mg/m3 is recommended. A short-term exposure limit (STEL), for workers, was developed reative to that for GB. The recommended value is 0.00004 mg/m3, for up to four 15-minute exposures per day. Similarly, relative to those for GB, acute exposure guideline levels (AEGL-1) were developed for the general population for exposure durations of 30 minutes, 60 minutes, and four hours. The recommended concentrations are 0.00024 mg/m3, 0.00012 mg/m3, and 0.00003 mg/m3.

Robineau 1987. Cardiac abnormalities in rats treated with methylphosphonothiolate. *Toxicol Appl Pharmacol.* 87(2):206-11.
Cardiac toxicity of methylphosphonothiolate (MPT), an organophosphorus compound, has been investigated in the rat. Subcutaneous injection of MPT (12 micrograms/kg body wt) induced cardiac arrhythmias, the occurrence of which was significantly more frequent than in the control group. Death rate among MPT-treated animals appeared to be in relationship with cardiac arrhythmias. Plasma nonesterified fatty acid concentrations increased in MPT-poisoned rats. cAMP and cGMP contents in myocardial tissue were unchanged 150 min after MPT administration, as compared with control. Similarly, no change has occurred in high energy compound levels.

Robineau et al. 1987. Effects of an organophosphorus compound on cardiac rhythm and hemodynamics in anaesthetized and conscious beagle dogs. *Toxicol Lett* 37:95-102.
Cardiac toxicity of S-(2-diisopropylaminoethyl)-O-ethylmethyl phosphonothiolate (VX) has been investigated in the dog. Conscious or open-chest anaesthetized animals were subcutaneously injected with VX (1.5, 3.0 or 6.0 micrograms/kg b.w.). Blood cholinesterase activity decreased to 60%, 20% and 18% respectively of initial values. Only in the 6.0 micrograms/kg-treated group, heart rate, arterial and left intraventricular pressures and contractility index slightly but significantly decreased. In some dogs, treated with either 3.0 micrograms/kg or 6.0 micrograms/kg b.w. of VX, the electrocardiogram was changed: the Q-T interval was lengthened and arrhythmias (atrioventricular blocks, ventricular premature complexes. 'Torsade de pointe') were observed. Plasma non-esterified fatty acid concentrations were identical in control and VX-poisoned dogs. This study shows that, besides the expected cardiac effects resulting from muscarinic stimulation, VX can affect ventricular function through a yet unknown mechanism.

Robineau et al. 1991. An organophosphorus compound, VX, selectively inhibits the rat cardiac Na+,K(+)-ATPase alpha 1 isoform. Biochemical basis of the cardiotoxicity of VX. *FEBS Lett*. 281(1-2):145-8.
Serine-specific reagents, anticholinesterase organophosphorus compounds like Vx provoke, in the micromolar range, digitalis-like ventricular arrhythmias of non-cholinergic origin in rodent hearts. The sensitivities of the two rat cardiac Na+,K(+)-ATPase isoforms (alpha 1 and alpha 2) to Vx (0.1-100 microM) were measured in sarcolemma vesicles. At 1 microM Vx, the inhibition of the total activity averaged 18% but never exceeded 75% with 100 microM. When the alpha 2 isoform activity was inhibited by 0.1 microM ouabain, alpha 1 was 35% inhibited by 1 microM Vx, i.e. a 16 +/- 4% inhibition of the total activity. The cardiac alpha 1 being related to the digitalis-induced toxicity, its selective inhibition by a micromolar dose of Vx fully accounts for the cardiotoxicity of Vx. Inasmuch as Vx had no effect on the rat kidney alpha 1, differentially inactivated the cardiac isozymes and specifically reacted with serine residues, the putative binding-site(s) of the organophosphorus compound on the Na+-K(+)-ATPase molecules has been considered.

Sidell et al. 1974. The reactivatibility of cholinesterase inhibited by VX and sarin in man. *Toxicol. Appl. Pharmacol*.27(2): 241-252.
The cholinesterase inhibitors VX (S,S-(2-diisopropylaminoethyl) O-ethyl methyl phosphonothiolate) and sarin (isopropyl methyl phosphonofluoridate) were given to normal subjects. An iv dose of 1. 5 mug/kg and an oral dose of 4.0 mug/kg of VX caused a 75% inhibition of erythrocyte cholinesterase. The oxime 2-pyridinium aldoxime methochloride (pralidoxime, 2-PAMCl) was administered at varying times and over a range of doses to these subjects who had received VX and also to subjects who had received sarin. It was found that the RBC-ChE inhibited by VX spontaneously reactivates much faster than that inhibited by sarin. VX-inhibited RBC-ChE ages very little and is amenable to oxime reactivation for as long as 48 hr; the dose of oxime necessary to reactivate VX-inhibited RBC-ChE is less than that needed to reactivate sarin-inhibited enzyme.

Sidell 1997. Nerve Agents. In *Medical Aspects of Chemical and Biological Warfare*. Chapter 5. Eds. Sidell FR, Takafuji ET, Franz DR.. Office of the Surgeon General, Dept. of the Army, United States of America.
Available at http://www.bordeninstitute.army.mil/cwbw/default_index.htm.

Sidell et al. 1997. Longterm Health Effects of Nerve Agents and Mustard. In *Medical Aspects of Chemical and Biological Warfare*. Chapter 8. Eds. Sidell FR, Takafuji ET, Franz DR.. Office of the Surgeon General, Dept. of the Army, United States of America.
Available at http://www.bordeninstitute.army.mil/cwbw/default_index.htm.

Smart 1997. History of Chemical and Biological Warfare: An American Perspective. In *Medical Aspects of Chemical and Biological Warfare*. Eds. Sidell FR, Takafuji ET, Franz DR. 1997. Office of the Surgeon General, Dept. of the Army, United States of America.
Available at http://www.bordeninstitute.army.mil/cwbw/default_index.htm.

Somani et al. 2001. Low-Level Nerve Agent Toxicity under Normal and Stressful Conditions. In *Chemical Warfare Agents: Toxicity at Low Levels.* Chapter3. Eds. Somani SM, Romano JA. CRC Press Boca Raton.

Special Assistant to Undersecratary of Defense for Gulf War Illnesses, Medical Readiness, and Miltary Deployment. 2004. *Project Shipboard Hazard and Defense (SHAD) Flower Drum Phase II* available at http://deploymentlink.osd.mil/pdfs/flower_drum_phase_ii.pdf

Special Assistant to Undersecratary of Defense for Gulf War Illnesses, Medical Readiness, and Miltary Deployment. 2004. *Project Shipboard Hazard and Defense (SHAD) Fearless Johnny* available at http://deploymentlink.osd.mil/pdfs/fearless_johnny.pdf .

Tsuchihashi et al. 1998. Identification of Metabolites of Nerve Agent VX in Serum Collected from a Victim. *J. Anal. Toxicol.* 22:383-388.
A human serum sample collected from a victim of the Osaka VX incident was analyzed according to our developed technique for metabolites of VX. Gas chromatography-mass spectrometry (GC-MS) in full-scan electron impact and chemical ionization modes were used, and, for more reliable confirmation, GC-MS-MS was also employed. In the serum sample, both ethyl methylphosphonic acid and 2-diisopropylamino-ethyl)methyl sulfide were detected. These results indicated that the techniques using GC-MS and GC-MS-MS were applicable to biological samples such as serum. These results also provide the first documented, unequivocal identification of the specific metabolites of VX in victim's serum and, furthermore, clarify a part of the metabolic pathway of VX in the human body.

Tuovinen 2004. Organophosphate-induced convulsions and prevention of neuropathological damages. *Toxicology.* 196:31-9.
Such organophosphorus (OP) compounds as diisopropylfluorophosphate (DFP), sarin and soman are potent inhibitors of acetylcholinesterases (AChEs) and butyrylcholinesterases (BChEs). The acute toxicity of OPs is the result of their irreversible binding with AChEs in the central nervous system (CNS), which elevates acetylcholine (ACh) levels. The protective action of subcutaneously (SC) administered antidotes or their combinations in DFP (2.0 mg/kg BW) intoxication was studied in 9-10-weeks-old Han-Wistar male rats. The rats received AChE reactivator pralidoxime-2-chloride (2PAM) (30.0 mg/kg BW), anticonvulsant diazepam (2.0 mg/kg BW), A(1)-adenosine receptor agonist N(6)-cyclopentyl adenosine (CPA) (2.0 mg/kg BW), NMDA-receptor antagonist dizocilpine maleate (+-MK801 hydrogen maleate) (2.0 mg/kg BW) or their combinations with cholinolytic drug atropine sulfate (50.0 mg/kg BW) immediately or 30 min after the single SC injection of DFP. The control rats received atropine sulfate, but also saline and olive oil instead of other antidotes and DFP, respectively. All rats were terminated either 24 h or 3 weeks after the DFP injection. The rats treated with DFP-atropine showed severe typical OP-induced toxicity signs. When CPA, diazepam or 2PAM was given immediately after DFP-atropine, these treatments prevented, delayed or shortened the occurrence of serious signs of poisoning. Atropine-MK801 did not offer any additional

protection against DFP toxicity. In conclusion, CPA, diazepam and 2PAM in combination with atropine prevented the occurrence of serious signs of poisoning and thus reduced the toxicity of DFP in rat.

Van der Schans et al. 2003. Toxicokinetics of the nerve agent (+/-)-VX in anesthetized and atropinized hairless guinea pigs and marmosets after intravenous and percutaneous administration. *Toxicol Appl Pharmacol.* 191(1):48-62.
In continuation of our investigations on the toxicokinetics of the volatile nerve agents C(+/-)P(+/-)-soman and (+/-)-sarin, we now report on the toxicokinetics of the rather nonvolatile agent (+/-)-VX. A validated method was developed to determine blood levels of (+/-)-VX by means of achiral gas chromatography at blood levels > or =10 pg/ml. The ratio of the two enantiomers of VX in blood could be measured at levels > or =1 ng/ml by using chiral HPLC in combination with off-line gas chromatographic analysis. In order to obtain basic information on the toxicokinetics of (+/-)-VX, i.e., under conditions of 100% bioavailability, the blood levels of this agent were measured in hairless guinea pigs at iv doses corresponding with 1 and 2 LD50. The derived AUCs indicate a reasonable linearity of the toxicokinetics with dose. Also, the toxicokinetics in marmoset primates was studied at an absolute iv dose corresponding with 1 LD50 in the hairless guinea pig which led to approximately the same levels of (+/-)-VX in blood as observed at 2 LD50 in the hairless guinea pig. Finally, the toxicokinetics of (+/-)-VX were measured in hairless guinea pigs via the most relevant porte d' entree for this agent, which is the percutaneous route at a dose corresponding with 1 LD50 (pc). Large variations were observed between individual animals in the rate of penetration of (+/-)-VX and in concomitant progression of AChE inhibition in blood of these animals. Blood levels of (+/-)-VX increased gradually over a 6-h period of time. After a 7-h penetration period, the total AUC corresponded with 2.5% bioavailability relative to iv administration. In contrast with the G-agents C(+/-)P(+/-)-soman and (+/-)-sarin, stereospecificity in the sequestration of the two enantiomers of (+/-)-VX is not a prominent phenomenon. It appears that (+/-)-VX is substantially more persistent in vivo than the two G-agents. This persistence may undermine the efficacy of pretreatment with carbamates of percutaneous intoxication in particular due to gradual replacement of carbamate on AChE by (+/-)-VX, whereas classical treatment of intoxication with oximes is hampered by the short persistence of oximes relative to the agent.

Van Der Schans et al. 2004. Retrospective detection of exposure to nerve agents: analysis of phosphofluoridates originating from fluoride-induced reactivation of phosphylated BuChE. *Arch Toxicol.* 78(9):508-24.
The utility was explored of a new approach to detect retrospectively exposure to nerve agents by means of conversion of the inhibitor moiety bound to the active site of the enzyme BuChE in plasma with fluoride ions into a phosphofluoridate which is subsequently analyzed by means of gas chromatography (GC). This quantifies >/=0.01% inhibition of BuChE and identifies the structure of the inhibitor except for the original leaving group. A three-tiered approach was followed involving the five classical nerve agents GA, GB, GF, GD, and VX, as well as the active metabolite of parathion, i.e., paraoxon: in vivo experiments in rhesus monkeys after iv administration of a sign-free dose of agent and concomitant in vitro experiments in plasma of rhesus monkeys and

humans should allow an assessment of in vivo retrospectivity in humans. A systematic investigation was performed in order to find a single set of reaction conditions which yields a maximum amount of phosphofluoridate for all nerve agents. Fluoride-induced reactivation at 25 degrees C at a final concentration of 250 mM KF during 15 min in a pH-range between 4 and 6 appears to be effective. The in vitro decrease with time in reactivatibility of inhibited BuChE in plasma from humans and rhesus monkeys was largely due to aging of the phosphyl moiety, except for VX where spontaneous reactivation was a major cause. The decrease followed first-order except for a biphasic course in the case of GF in human and rhesus monkey plasma as well as of GD in rhesus plasma. In vitro half-lifes in human plasma ranged between ca. 14 h for GB and ca. 63 h for GA. A comparison of the in vivo data from rhesus monkeys and the in vitro data is complicated by the observation that the in vivo decrease with time of fluoride-reactivated phosphofluoridate is biphasic for all nerve agents. The terminal in vivo phase pertains to a small fraction of the amount of initially regenerated phosphofluoridate but is responsible for a considerable degree of retrospectivity, ranging between 14 and 56 days for GF and GB, respectively. The new procedure can be used in a variety of practical applications, e.g., (i) biomonitoring in health surveillance at exposure levels that are several orders of magnitude lower than presently possible; (ii) diagnosis in case of alleged exposure to nerve agents in time of war or after terrorist attacks; (iii) in forensic cases against suspected terrorists that have handled organophosphate anticholinesterases; and (iv) in research applications such as investigations on lowest observable effect levels of exposure to nerve agents.

Vranken et al. 1982. In vitro inhibition of neurotoxic esterase by organophosphorus nerve agents. *Arch Int Pharmacodyn Ther.* 260(2):316-8.

Wester et al. 2000. Predicted chemical warfare agent VX toxicity to uniformed soldier using parathion in vitro human skin exposure and absorption. *Toxicol Appl Pharmacol.* 168(2):149-52.
Chemical warfare agents (CWA) are easily and inexpensively produced and are a significant threat to military forces and the public. Most well-known CWAs are organophosphorus compounds, a number or which are used as pesticides, including parathion. This study determined the in vitro percutaneous absorption of parathion as a CWA simulant through naked human skin and uniformed skin (dry and sweated). Parathion percentage dose absorbed through naked skin (1.78 +/- 0. 41) was greater than dry uniformed skin (0.29 +/- 0.17; p = 0.000) and sweated uniformed skin (0.65 +/- 0.16; p = 0.000). Sweated and dry uniformed skin absorption were also different (p = 0.007). These relative dry and sweated uniformed skin absorptions were then applied to VX skin permeability for naked skin (head, neck, arms, and hands) and the remaining uniformed skin over the various regions of the human body. Risk assessment shows VX 50% lethality within the first hour for a soldier wearing a sweated uniform. By 8 h postexposure to naked skin plus trunk area predicted lethality for both dry and sweated uniform, and, at 96 h postexposure, all body regions individually exposed would produce lethality. Military uniform and public clothing provide some immediate protection but absorption through cloth and skin does occur. Immediate safety response to skin and clothing is required.

Wetherell et al. 2002. Physostigmine and hyoscine improves protection against the lethal and incapacitating effects of nerve agent poisoning in the guinea-pig. *Neurotoxicology.* 23(3):341-9.
This study is drawn from a work programme aimed at developing improved medical counter measures for nerve agent poisoning. Guinea-pigs were administered pyridostigmine (5.1 microg/h) or physostigmine (4.7 microg/h) and hyoscine (1.94 microg/h) for 6 days via a subcutaneously implanted mini osmotic pump. Pyridostigmine inhibited red cell acetylcholinesterase (AChE) by 44.2 +/- 2.7% and plasma cholinesterase (ChE) by 29.9 +/- 1.8%. Physostigmine and hyoscine inhibited red cell AChE by 18.7 +/- 3.7% and plasma ChE by 44.1 +/- 3.1%. On day 6, animals were challenged with a lethal dose of tabun (GA; 125 microg/kg), sarin (GB; 51.2 microg/kg), soman (GD; 31.2 microg/kg), GF (50 microg/kg) or VX (11.25 microg/kg) administered by the subcutaneous route. Animals were closely observed for signs of poisoning. The time to the onset of signs of poisoning was similar for all the agents except for VX, which showed a delay compared to the other agents. Following pretreatment with either pyridostigmine or physostigmine and hyoscine most animals survived for 2-3 h following nerve agent administration. In contrast, only physostigmine and hyoscine prevented or reduced the duration of the signs of incapacitation and the temperature drop produced by all the agents. Pyridostigmine-pretreated animals showed little or no recovery from incapacitation prior to death. Physostigmine and hyoscine pretreatment provided statistically ($P < 0.05$) better protection against GB, GD and VX lethality (24 h) than pyridostigmine pretreatment and better protection against GA and GF lethality.