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HEALTH EFFECTS OF PROJECT SHAD CHEMICAL AGENT:

SARIN NERVE AGENT

[CAS # 107-44-8]

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This report and any supplements were prepared by the Center for Research Information, Inc. which is solely responsible for its contents.

Although this draft is the definitive submission on its subject matter, the Center for Research Information recognizes its ethical and contractual obligation to update, revise, or otherwise supplement this report if new or necessary information on its subject matter should arise, be requested, or be ascertained during the contract period.

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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

In 1936 German chemist Gerhard Schrader discovered that an organophosphate compound, ethyl dimethylphosphoramidocyanidate (later called Tabun), was a potent insecticide. Dr. Schrader reported his discovery to German authorities, who then set up a laboratory for Schrader to further pursue toxic nerve agents for military purposes. In 1938, Schrader along with some associates, synthesized 1-methylethyl methylphosphonofluoridate. It was named sarin, after the chemists **S**chrader, **A**mbrose, **R**üdiger and van der L**in**de, who were responsible for its synthesis.

Sarin is a chemical warfare nerve agent which is described by the chemical formula $C_4H_{10}FO_2P$, and is identified by Chemical Abstracts Service (CAS) Registry number 107-44-8. Under normal conditions it is a colorless liquid, and odorless. It is miscible in both polar and nonpolar solvents, and it hydrolyzes slowly in water at neutral or slightly acidic pH. Sarin is significantly less stable to hydrolysis than VX. Sarin's hydrolysis products are considerably less toxic than the original agent.

The synthesis of sarin's chemical class, the organophosphates, dates back to 1820. Widespread poisoning by organophosphates was first seen in the United States in early 1930, when many people developed a strange paralytic illness traced to a Prohibition-era alcohol substitute, called Jamaican Ginger or Jake, which had been adulterated with tri-ortho-cresyl phosphate (TOCP). TOCP was the first chemical proven to show a delayed type of neurotoxicity.

The use of chemical warfare agents is ancient but their most extensive use occurred during World War I when chlorine and mustard gas inflicted over one-million casualties. Nazi Germany later produced large amounts of the organophosphate agent tabun along with far lesser amounts of sarin (1000 lb) throughout World War II but they were not known to be used. In 1950, the US Army's Chemical Corp began the construction of plants for the full-scale production of sarin but ceased in 1957 because stockpile requirements were met.

The only confirmed military use of nerve agents in history was by Iraq, which used tabun and sarin aerial bombs to repel Iranian troops. In the latter part of the war, Iraq's extensive use of chemical warfare agents is believed to have brought an end to the conflict. Reports claim that between 5,500 to 10,000 Iranian troops were killed by nerve agents and mustard gas, and up to 100,000 soldiers were exposed. In March of 1988, Iraq used a combination of chemical weapons, including mustard gas, tabun, sarin, VX and possibly even cyanide to kill as many as 5,000 people in the Kurdish town of Halabja. Iraq is believed to have produced between 790 to 810 tons of sarin which degraded or were destroyed after the Gulf War.

The first known terrorist use of a nerve agent involved sarin and occurred in Matsumoto City, Japan on the evening of June 27, 1994. About 12 liters of sarin were released using

a heater and a fan from the window of a delivery truck. The attack was undertaken to kill four judges involved in a dispute with the Aum Shinrikyo cult. There were 471 victims of sarin poisoning, 54 were hospitalized, and 253 treated at outpatient facilities. Seven died. On March 20, 1995, Aum Shinrikyo launched an even bolder attack on the subway system in Tokyo. At 8:00 AM, at the height of rush hour, sarin was released. Twelve subway passengers were killed. About 980 persons suffered mild to moderate exposure, and 500 persons were hospitalized. Over 5,000 people, many of whom were not actually exposed, sought medical attention.

The largest experimental use of sarin on humans appears to have occurred at Porton Down in the United Kingdom in the 1950s. The purpose of the studies was to obtain precise information on the toxic properties of these agents. Certain experiments went terribly wrong. One man died 45 minutes after 200 mg of sarin were dripped onto a uniform patch on his forearm.

The US also ran a number of tests using sarin that may have resulted in human exposure. The tests were part of Project 112 of the Deseret Test Center; Project SHAD (Shipboard Hazard and Defense) was part of this program. The tests monitored the environmental effects of sarin, the dispersal pattern of bomblets, shipboard detection of agents, and protective measures. Several of the tests did involve exposure of personnel to nerve agents and to potential biowarfare agents. The Department of Defense (DOD) has identified about 5,000-6,000 persons who may have been exposed to one or several of these agents.

Very little data on Soviet chemical weapons testing has emerged. Several reports indicate that there was exposure of the population in Russia to nerve agents. One paper had a short summary reporting 209 acute poisonings involving sarin, soman or VX in Russian production facilities. Several long term health effects were described including memory loss, asthenia, sleep disorders and cardiovascular effects.

The most widespread use of nerve agents occurred during and shortly after the Iran-Iraq war but unfortunately there is very little accessible scientific literature addressing either the short term or long term medical consequences of this use. Iraq used nerve agents and mustard gas against its Kurdish population from April 1987 to October 1988, to quell rebellion and punish the population. It is estimated that approximately 250,000 civilians where exposed to these agents and over 5,000 were killed. Unfortunately, there also been very little study of this population. The exposures to nerve agents in Japan remain the most extensively studied sarin incidents.

The acute toxicity of sarin is believed to be rooted in its inhibition of acetylcholinesterases (AChE's). The inhibition of AChE leads to a rise in the concentration of acetylcholine and the hyperstimulation of both nicotinic and muscarinic acetylcholine nerve receptors. Sarin has been shown to react with a number of other receptors and enzymes as well. At very low concentrations (0.3-1.0 nM), sarin reacts with muscarinic m2 receptors on presynaptic gamma-aminobutyric acid (GABA)-ergic neurons. The reduction in the action-potential mediated release of GABA can account for

the occurrence of seizures in individuals exposed to sarin. Sarin also bind tightly to muscarinic m2 receptors in the heart and may play a role in cardiotoxicity.

There have been several reports on the ability of sarin to inhibit the enzyme neurotoxic esterase or neuropathy targeted esterase (NTE). The inhibition of NTE has been reported to be responsible for the onset of organophosphate induced delayed neuropathy (OPIDN). The pathway through which inhibition of NTE leads to OPIDN has not yet been elucidated, although it is known neuropathy only occurs when over 70% of NTE activity is inhibited following acute exposure and 50% following repeated exposures. It should be noted that subclinical neuropathy was reported 30 days after sarin exposure in Japan and a subsequent study also picked up electromyographic evidence of neuropathy six months after exposure.

The acute effects of sarin are believed to be primarly due to (-)-isomer of sarin. The (+)-isomer appears to be eliminated rapidly from body following administration. Animal studies indicate that (-)-sarin is rapidly distributed throughout the body, within minutes, but eliminated very slowly with a half-life of several hours. The primary metabolite of sarin, isopropyl methylphosphonic acid, was found in large amounts in the serum and urine of victims in Japan. The concentration of the metabolite and the amount of time from exposure can be used to estimate the level of exposure. These studies indicated that several of the survivors were exposed to supra-lethal levels of sarin.

There are currently no real time clinical tests for Sarin exposure but there have been a number of forensic assays developed that can confirm exposure. Most of these tests involve isolating RBC AChE and/or serum butyrylcholinesterase from blood and releasing and detecting any organophosphates that are released. There has also been a great deal of work on the environmental detection of sarin and other nerve agents. The Department of Defense has developed several detectors to monitor air for the presence of nerve agents. The mainstay of the Army chemical detection is the M8A1 alarm which constantly samples the air for higher molecular weight molecules. The detector ionizes gases and mass filters away the low molecular ions generated from air.

The health effects of sarin, are dependent on the route of administration, dose received, and the speed at which treatment is given. Casualties can go from being fully functioning to comatose with severe respiratory distress in a matter of seconds following exposure. Aggressive, rapid therapy can substantially minimize adverse health effects seen in patients exposed to nerve agents.

Acute effects seen at low concentrations include: miosis, ocular pain, blurred or dimmed vision, tearing, rhinorrhea, broncospasm, slight dyspnea, respiratory secretions, salvation, and diaphoreses. At intermediate concentrations, moderate dyspnea, nausea, vomiting, diarrhea are seen. At high concentrations, convulsions, loss of consciousness, muscle fasciculations, flaccid paralysis, copious secretions, apnea and death.

Toxic factors and exposure limits established by the US Dept. of Health and Human Services (DHHS) include the vapor concentration per period of exposure during which

50% lethality is seen for humans (LCt_{50}). That level is 100 mg/m^3/min; the no death dose equals 10 mg/m^3/min; the no neuromuscular (NNM) effect dose equals 4 mg/m^3/min. The concentration which induces miosis in 50% of victims (ECt_{50} (miosis)) equals 2-4 mg/m^3/min. The no observable effect level (NOEL) equals 0.5 mg/m^3/min; the maximal single concentration for 1 hour equals 0.001 mg/m^3; the maximal single concentration for 8 hours equals 0.0003 mg/m^3; the safety factor of 0.1 is used for the general population and the limit levels are 0.0001 mg/m^3 for 1 hour, 0.00003 mg/m^3 for 8 hours and 0.000003 mg/m^3 for 72 hours.

There has been no evidence in humans of reproductive or developmental toxicity. In animals, there has been no evidence of sarin related adverse effects with respect to reproductive performance, fetal toxicity, and teratogenesis. There is no evidence of carcinogenicity in human. In chronic inhalation studies in mice, rats and dogs, sarin did not appear to be carcinogenic. No significant pulmonary tumors were observed in strain A mice after 3/19 and 3/20 animals after 52 weeks of exposure to 0.001 and 0.0001 mg/m^3, respectively.

There is relatively little information available regarding the human genotoxicity of sarin. In bioassays using bacteria and mammalian cell cultures with and without metabolic activation sarin did not show any evidence of genotoxic or mutagenic activity. There was no increase in mutations using the Ames test. But several studies of the victims of the Tokyo subway attack indicate that the sister-chromatid exchange (SCE) of lymphocytes was higher in persons exposed to sarin, and there was a positive correlation between the extent of serum cholinesterase inhibition and the level of SCE. The SCE effect appeared to last up to three years after exposure.

Miosis (pinpoint pupils) is characteristic of sarin exposure. It usually occurs within seconds or minutes of exposure. It can last up to 9 weeks resulting in dim vision. Blurred vision and eye pain can accompany sarin exposure. There is very little data on the effect of sarin on hearing.
Rhinorrhea, typically intense, is often seen shortly after sarin exposure. Tightness in the chest is a common symptom after exposure to small amounts of sarin and usually dissipates within hours of exposure. As the amount of exposure increases, dyspnea and pulmonary distress increase and often someone severely poisoned will go into respiratory failure and die. No data indicate that respiratory effects persist long after exposure.

Several animal studies that indicate there is a potential for some immunotoxicity or immunodulatory effects upon sarin exposure. Reductions of T-cell mediated immune reaction, a substantial increase in NK cell and macrophage activity, and a substantial decrease in CD4 T-cell activity have been seen in testing. A single exposure was observed to have the same effect as multiple exposures.

Bradycardia is frequently seen following moderate or high level sarin exposure. There have been reports of persistent arhythmias following exposure. In cases of severe poisoning, cardiomyopathy may also be seen.

Neuromuscular effects are common as acetylchloline is a primary neurotransmitter at the neuromuscular junction. Increased acetylcholine initially leads to stimulation, followed by fatigue and muscle paralysis. In the Tokyo attack, asthenia or muscle weakness was seen in most patients upon admission to the hospital. Following liquid exposure muscle fasciculations at the site of exposure are often seen after excessive sweating. Long-term shoulder stiffness may be a result of exposure. Myopathy has also been seen in rats in the absence of treatment following a moderate dose of sarin.

Although inducing convulsions and the resultant neuropathology, sarin in the the Japanese incidents was found not to have caused persistent neurological disorders in most patients. One exception was a patient who suffered from akinetic mutism for at least two years following exposure. Studies in rats have shown that there is wide variability in neurotoxicity following repeated sublethal doses of sarin. There is a lack of tolerance with repeated doses, and a cumulative effect on toxicity.

Headaches are a very common symptom of sarin exposure. Loss of memory can happen; a case of amnesia is reported following exposure in Japan. Long term changes in electroencephalograms (EEG) in workers following accidental sarin exposure have been observed. Increases in REM sleep have been found.

The initial diagnosis of sarin exposure is based on signs, symptoms and historical factors. The first step in the diagnosis is to confirm presence of both nicotinic and muscarinic effects. A convenient mnemonic for the signs and symptoms of nerve agent poisoning is dumbbels, which stands for **D**iaphoresis (and diarrhea); **U**rination; **M**iosis; **B**radycardia; **B**ronchospasm (and bronchorrhea); **E**mesis; **L**acrimation (with rhinorrhea and salivation); **S**eizures (as well as muscle fasciculation and weakness).

Symptoms depend on the site and extent of exposure. Following dermal contact symptoms can be delayed 18 hours but symptoms from inhalation can occur within seconds. Percutaneous absorption of liquid sarin also occurs readily and typically leads to localized sweating, followed by muscular fasciculations and weakness. (Lee 2003, National Research Council 1997). Useful markers of nerve agent exposure include serum butyrylcholinesterase and red blood cell AChE activity. Significantly reduced levels of these are indicative of nerve agent exposure. Analysis of patients from the Tokyo subway event indicates that miosis may be a better indicator of potential systemic toxicity than red blood cell (RBC) AChE levels.

Psychogenic effects were reported from the Japanese incidents. Post-traumatic stress disorder (PTSD) was seen in a number of sarin victims. Several studies have shown persistent decreases in serum cholinesterase activity in patients with PTSD that evolved over six months with no correlation with the serum cholinesterase activity taken right after exposure. Fatigue, asthenia, insomnia, blurred vision, general anxiety were the common manifestations. A survey of general effects of perceived exposure to chemical and biological warfare agents is contained in the supplement under this contract "Psychogenic Effects of Perceived Exposure to Biochemical Warfare Agents."

There are essentially five components of treatment for sarin exposure. The first component is prophylaxis. This is typically accomplished by the administration of pyridostigmine, a carbamate that reacts reversibly with AchE protecting the enzyme from inactivation. The second component of treatment is decontamination and evacuation. The third component of treatment is the use of anticholinergic agents to block the effect of increased acetylcholine at synapses. Atropine is commonly used for this purpose. The fourth component is the use of oximes to regenerate AchE enzymes. The fifth component of treatment is the use of anticonvulsants. Diazepam has been the mainstay of anticonvulsant therapy for nerve agent poisoning. In addition to these treatments, there has also been interest in using adenosine agonists such as N6-cyclopentyladenosine (CPA) to attempt to decrease the amount of acetylcholine released at synapses. CPA has shown promise in reducing the potential cardiovascular toxicity following sarin exposure.

Future study of sarin would benefit from greater availability and evaluation of sarin-exposure and testing data from the Iran-Iraq war and the former Soviet Union.

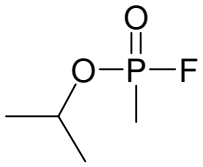
II. BACKGROUND: CHEMISTRY & HISTORY

Chemistry

Project SHAD Agent name: Sarin

Formula: $C_4H_{10}FO_2P$

Structure:



CAS Number: 107-44-8

Names:
Sarin
Phosphonofluoridic acid, methyl-, 1-methylethyl ester
Phosphonofluoridic acid, methyl-, isopropyl ester, (+-)-
T-144
T-2106
TL 1618
Trilone 46
IMPF
Isopropoxymethylphoshoryl fluoride
Isopropoxymethylphosphoryl fluoride
Isopropyl methanefluorophosphonate
(+-)-Isopropyl methylphosphonofluoridate
GB
o-Isopropyl methylphosphonofluoridate

Physical Properties (Winkenwerder 2002a)
Colorless liquid
Miscible in water
Odorless and stable when pure
Nonflammable
Molecular Weight 140.1
Vapor Density (Air = 1) = 4.86
Liquid Density (g/cc) = 1.0887 @ 25° C
Freezing/Melting Point = -56° C
Boiling Point = 158° C
Vapor Pressure (mm Hg) = 2.9 @ 25° C; 2.1 @ 20° C
Log K_{ow}= 0.72

Decomposition Temp. = 150° C

Sarin is a chemical warfare nerve agent.

Sarin is miscible in both polar and nonpolar solvents. It hydrolyzes slowly in water at neutral or slightly acidic pH and more rapidly under strong acid or alkaline conditions. Sarin is significantly less stable to hydrolysis than VX, particularly at acidic pH. The hydrolysis products are considerably less toxic than the original agent (HSDB 2004). Sarin is metabolized by both paraoxonase (PON1) and butyrylcholinesterase (also known as serum acetylcholine esterase). The P-F linkage is hydrolyzed, resulting in the formation of a relatively non-toxic phosphonic acid (Costa et al. 1999, Raveh et al. 1993)

Sarin is also a racemic mixture of stereoisomers, the chiral center of the molecule is on the phosphorus atom. The (-) isomer of sarin appears to react with acetylcholinestrase (AchE) at a rate at least 3-4 orders of magnitude greater than the (+)-isomer. The (+)-isomer is referred to as the non-toxic isomer but may convert to (-)-isomer in-vivo. It should be noted that it has been difficult to obtain (+)-isomer in pure form (Benschop et al. 2001, Spruit et al. 2000)

History of Development & Use as Weapon

The use of chemical warfare agents goes back to antiquity. Early documented uses of chemical warfare agents include: the Chinese use of arsenical smoke circa 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 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 chlorine and mustard gas attacks resulted in over one-million casualties (Eckert 1991, Landersman 2003, Smart 1997).

The synthesis of organophosphosphates dates back to 1820, when Jean Louis Lassaigne reacted phosphoric acid with alcohols (Furtado et al. 2003). The first reported toxicity involving organophosphates happened in France, when creosote oil was used to treat tuberculosis and several of the patients developed neuromuscular spasms. The US experienced widespread poisoning involving organophosphates in the early 1930s, when many in the Midwest and South developed a strange paralytic illness that eventually resulted in spastic gaits. Dr. Maurice Smith of the National Institutes of Health (NIH) traced the disease to an alcohol substitute called Jamaican Ginger, or Jake, that had been adulterated with tri-ortho-cresyl phosphate (TOCP). (TOCP was also the first chemical proven to exhibit a delayed-type of neurotoxicity.)

Approximately 20,000-50,000 people were poisoned in the Jamaican Ginger incident. Some recovered from the syndrome but for many the damage was permanent. The characteristic gait was referred to as Jake leg and inspired a number of popular songs (Smith 1930, Parascandola 1995, Morgan 1978, Ecobichon 2001, Baum 2003).

In 1936, Gerhard Schrader, a German chemist working for I.G. Farben, discovered that an organophosphate compound named ethyl dimethylphosphoramidocyanidate (later called Tabun) was a potent insecticide. During the course of their experiments, both Dr. Schrader and an assistant were exposed to Tabun and suffered untoward effects, including miosis and shortness of breath. As required by German law, Dr. Schrader reported his discovery to German authorities who requested a demonstration. They then set up laboratory for Schrader to further pursue toxic nerve agents for military purposes (Smart 1997; Mitretek 2004).

In 1938, Schrader along with associates synthesized 1-methylethyl methylphosphonofluoridate. The compound was named sarin after the chemists **S**chrader, **A**mbrose, **R**üdiger and van der L**in**de who were responsible for its synthesis. Sarin was significantly more toxic than Tabun. Although Germany produced large amounts Tabun (12,000 tons) and smaller amounts of Sarin (1000 lb) during World War II, neither of the agents appears to have used during the war (Smart 1997; Mitretek 2004).

After World War II, the US Army's Chemical Corp began the construction of plants for the full-scale production of sarin. The Army developed a five-step process for synthesis that was spread across two plants. The first two steps were performed at a plant at Muscle Shoals, Alabama; the final steps were completed at Rocky Mountain Arsenal, Colorado. Plant construction was completed and production began in 1953. In 1957, the Army ceased production because stockpile requirements were met and the Army decided to move forward with VX production. The Army also developed a number of munitions to deliver sarin, including cluster bombs, large bombs, 105 mm and 155 mm artillery shells, and rockets with warheads capable of delivery over very long distances (Smart 1997).

Although the US Army has never used nerve agents in war, there have been both accidental and intentional releases that have resulted in the exposure of the environment, soldiers, and civilians. Operation Chase involved the disposal of the M55 rocket system after the thin aluminum heads of the rockets which contained the sarin began to leak. In 1967, the Army decided to dispose of the leaking munitions by encasing them inside concrete within ships, and then the sinking the ships. The dumping of sarin filled rockets into the sea raised serious concerns among the public. Another well-known incident occurred in Okinawa on July 8, 1969 when an accident occurred during the cleaning of sarin or VX filled shells. Twenty-three soldiers and one civilian were exposed. Although there were no fatalities, this event created an international incident when Japan demanded that chemical weapons be removed from the island (Smart 1997).

In the late 1960s, it was also revealed that there had been open air testing of nerve agents both at Edgewood and in Hawaii during 1966-67. These incidents, along with an incident in Britain which involved the release of VX, turned the public against chemical weapons. In 1969, President Nixon renounced the first strike use of chemical weapons and curtailed research on chemical agents (Smart 1997).

Nerve agents were also tested in Panama, particularly between 1964-1968, when the US Army test fired chemical munitions both in the Canal Zone and on San Jose Island. It is not known if there was any human exposure during these tests (**Lindsay-Poland** 1998).

Although the Army Chemical Corp was not eliminated during the 1970s, its activities were diminished. Research nevertheless continued on binary weapons, which are weapons in which two less toxic and more stable chemicals mix upon firing to form a nerve agent. (Munitions containing phosphonic difluoride in one canister and isopropanol and isopropylamine in the other formed sarin upon firing.) The Army Chemical Corp was rejuvenated during the 1980's, in response to a perceived gap in chemical warfare agents between the US and the former Soviet Union (Smart 1997).

During the 1980s, the only confirmed military use of nerve agents occurred when Iraq used tabun and sarin-filled aerial bombs to repel Iranian troops in the Iran-Iraq war. (Iraq's chemical weapons program dates back to 1971, when a small facility was built at Rashid.) Iraq continued to produce tabun, VX, and sarin throughout the 1980s. Iraq's extensive use of chemical warfare agents is believed to have brought an end to the conflict. Reports claim that between 5500 to 10,000 Iranian troops were killed by nerve agents and mustard gas, and that up to 100,000 soldiers were exposed (Peterson 2002, Federation of American Scientists 1990).

Iraq was also the only government to use nerve agents to quell civilian uprisings. In March of 1988, Iraq used a combination of chemical weapons, including mustard gas, tabun, sarin, VX and possibly even cyanide to kill as many as 5000 people in the Kurdish town of Halabja. Iraq is believed to have ultimately produced between 790 to 810 tons of sarin. The sarin was of low quality and purity, however, and could only be stored for limited periods of time. During 1992-1994, UN weapons inspectors destroyed 70 tons of sarin in Iraq (Federation of American Scientists 2004; Council on Foreign Relations 2004). A recent reminder of the Iraq Chemical Weapons program was the discovery of a binary weapon that designed produced sarin in an improvised explosive device near Baghdad. The shell was believed to be a remnant from a test firing from the earlier period that had failed to explode (Ritter 2004).

The first known terrorist use of a nerve agent involved sarin and occurred in Matsumoto City, Japan on the evening of June 27, 1994. During this attack, about 12 liters of sarin were released using a heater and a fan from the window of a delivery truck. The attack was undertaken to kill four judges involved in a dispute with the Aum Shinrikyo cult. This attempt resulted in 471 victims of sarin poisoning. Fifty-four were hospitalized, 253 were treated at outpatient facilities and 7 died. All of the judges survived (Nakajima 1998; Organisation for the Prohibition of Chemical Weapons (OPCW) 2004).

On March 20, 1995, Aum Shinrikyo launched an even bolder attack, this time on the subway system in Tokyo. In this attack 5 two-person teams, consisting of a subway rider and getaway driver, worked together. The target station was Kasumigaseki, near a large number of government buildings and the headquarters of the Tokyo police. Each of the subway riders executing the attack carried several double layered plastic bags containing

approximately 20 ounces of sarin. At 8:00 AM, at the height of rush hour, each subway rider pierced the plastic bag and exited the train. Twelve subway passengers were killed in these attacks, 980 persons suffered mild to moderate exposure, and 500 persons were hospitalized. Over 5000 people nonetheless sought medical attention; many of these had not even been exposed (Organisation for the Prohibition of Chemical Weapons (OPCW) 2004).

The US and 161 other countries have joined the Chemical Weapons Convention (CWC), in which they pledged not to develop or stockpile chemical weapons. The CWC created the Organisation for the Prohibition of Chemical Weapons (OPCW) which is responsible for implementing the convention. The countries that are signatories have pledged to destroy the existing stockpiles of chemical weapons by the year 2007. As of April 2004, roughly 12% of the world stockpile has been destroyed. In the year 2000, there were over 15,000 tons of sarin declared by several countries (Bismuth et al. 2004, CWC 2004A, CWC 2004B).

Study & Reports of Human Exposure

Although nerve agents were invented in Nazi Germany, the extent and type of human testing during that period have not been directly reported (Augsberger 2000). The largest experimental use of sarin on humans appears to have occurred at Porton Down in the United Kingdom in the 1950's. An inquest into the death of Ronald Maddison revealed that as many as 3000 to 20,000 volunteers may have been exposed to sarin and other nerve agents between 1947 and 1989 (Barnett 2003, Edwards 2000).

The purpose of the studies was to obtain precise information on the toxic properties of nerve agents. Most of the volunteers were not told they were participating in studies on nerve agents but many were told the studies were to develop a cure for the common cold. In 1953, scientists at Porton Down were trying to determine the precise lethal dose of sarin. These particular studies involved 396 soldiers, who had various amounts of sarin dripped onto patches on their uniforms in a sealed gas chamber. The experiments went terribly wrong resulting in the death of one volunteer and at least the hospitalization of several others for extended periods of time. In the case of the fatality, 200 mg of sarin had been dripped onto a uniform patch on Mr. Maddison's forearm and 45 minutes later he was dead. The coroner's report was never released, but there is currently an inquest into the nerve agent experiments at Porton Down (Barnett 2003, Edwards 2000).

The US also ran a number of tests using sarin that may have resulted in human exposure. The tests were part of Project 112 of the Deseret Test Center; Project SHAD was part of this program. The tests monitored the environmental effects of sarin, the dispersal pattern of bomblets, shipboard detection of agents, and protective measures. Several of the tests did involve exposure of personnel to nerve agents and potential biowarfare agents. The Department of Defense (DOD) has identified about 5000-6000 persons who may have been exposed to one or several of these agents. Many aspects of these tests are

still classified, although the DOD did report that there were no acute illnesses reported during testing. At least 246 of these soldiers were exposed to sarin (Veterans Administration 2004, DeploymentLink 2003, Spencer et al. 2000). DOD has recently acknowledged that some civilians may have also been exposed during testing although there appears to be no health records of that exposure (Mientka 2002).

Sarin was introduced to the Soviet arsenal as early as 1946. Unfortunately, very little data on the Soviet chemical weapons testing have emerged. Several reports indicate that exposure to nerve agents of elements of the Russian population took place. Much of the information was not collected or has not been revealed. Russia declared a stockpile of over 11,000 tons of sarin on hand in the 1990s (Fedorov 1994).

One paper that has emerged gives a short summary of 209 acute poisonings involving sarin, soman or VX in Russian production facilities. Several long term health were described including memory loss, asthenia, sleep disorders and cardiovascular effects (Yanno et al. 1997)

The use of nerve agents during and shortly after the Iran-Iraq war produced very little accessible scientific literature addressing either the short term or long term medical consequences of the use. Nevertheless, a recent paper analyzed the work of an Iranian physician who treated a number of victims of nerve agent poisoning. In these papers, Iranian physicians emphasized the importance of evacuation, decontamination and aggressive early therapy in treating victims (Newmark 2004).

Iraq also used nerve agents and mustard gas against its Kurdish population from April 1987 to October 1988, to quell rebellion and punish the population. It is estimated that approximately 250,000 civilians where exposed to these agents and over 5,000 were killed. Unfortunately, there also been very little humanitarian aid or study of this population for either environmental or long-term health effects (Gosden 2002).

In contrast to the widespread exposures in Iraq and Iran that were poorly studied, exposures to nerve agents in Japan have been extensively studied. The two terrorist incidents in Japan perpetuated by the Aum Shinrikyo resulted in sarin exposure for several thousand people. (Seto 2001; Okudera et al. 1997; Morita et al. 1995). Approximately 5,500 passengers were exposed, 9 passengers and two station officers were killed. The two station officers died from touching the plastic bags. In addition to passengers and station workers, 135 of 1384 emergency workers and 23% of the hospital workers at St. Lukes, the hospital that received over 600 victims, displayed signs or symptoms of secondary exposure. None of the emergency or hospital workers died. If Aum Shinrikyo would have used a more concentrated solution the result may have been much worse (Okumura et al. 2000, Okumura et al.1998a, Okumura et al. 1998b, Yokohama et al. 1998).

In addition to intentional releases, there have been a number of accidental releases of sarin that resulted in human exposure. The largest exposure may have occurred after the Gulf War in Iraq when US soldiers destroyed munitions in Pit and Bunker 73 in

Khamisiyah Iraq. Bunker 73 was destroyed on March 4, 1991 and the Pit was destroyed on March 10, 1991. Reports indicated as much 8 tons of sarin/cyclosarin were present in approximately 1250 rockets that were destroyed in the Pit and in the additional munitions destroyed in the Bunker. Recently revised CIA/DOD estimates indicate that a worst case is that 321 kg of sarin/cyclosarin were released from the Pit demolition and that 51 kg were released from the destruction of Bunker 73. It is unlikely that any US ground were exposed following the demolition of Bunker 73 but it is likely that US ground forces were exposed to low levels of nerve agents following the demolition of the Pit. DOD has identified as many 100,000 troops who may have been in hazard areas and possibly exposed to low levels of nerve agents (Winkenwerder 2002b).

In 1979, Duffy reported on long-term effects of production workers, who were exposed to sarin. In his studies he identified 77 workers who were exposed to either low or moderate amounts of sarin at least once (Duffy et al. 1979). On July 14, 2002, four workers at Army's Chemical Depot in Tooele, Utah, were exposed to sarin during disposal operations. One of the workers suffered serious complications (Zacharias 2002).

III. PATHOGENESIS & FORESNIC DIAGNOSTICS

Pathophysiology

The acute toxicity of sarin is believed to be predominantly due to the inhibition of acetylcholinesterases (AChE's). The inhibition of AChE leads to a rise in the concentration of acetylcholine and to the hyperstimulation of both nicotinic and muscarinic acetylcholine nerve receptors. AChE is responsible for the hydrolysis of acetylcholine to produce acetic acid and choline. The hydroxyl group on a serine residue in the active site of the enzyme helps cleave the choline ester but is also phosphorylated by sarin. Once phosphorylated the reactivation of the enzyme is very slow and the enzyme-inhibitor complex can undergo a process called aging in which the enzyme is essentially irreversibly inactivated. The half-life of the aging process is about 5 hours. Once an enzyme has undergone aging it can no longer be reactivated by oximes such as pradiloxime chloride.(Spencer et al. 2000; Tripathi et al. 1989; Sidell et al. 1992).

In addition to AChE's, sarin has been shown to react with a number of other receptors and enzymes. Sarin reacts with muscarinic receptors in animal studies. At very low concentrations (0.3-1.0 nM), sarin reacts with muscarinic m2 receptors on presynaptic gamma-aminobutyric acid (GABA)-ergic neurons. This binding significantly reduced the postsynaptic current in GABA-ergic neurons. The reduction in the action-potential mediated release of GABA can account for the occurrence of seizures in individuals exposed to sarin (Chebabo et al. 1999).

Sarin has also been shown to bind tightly to muscarinic m2 receptors in the heart which may play a role in cardiotoxicity (Silveira et al. 1990). Sarin, unlike VX, appears to have no effect on the binding of alpha-cobrotoxin to nicotinic acetylcholine receptors (Chi et al. 1995). Single fiber electromyography studies in man have indicated that there is a down-regulation or desensitization of acetylcholine receptors at the neuromuscular junction following exposure to low levels of sarin. The down-regulation of acetylcholine receptors is believed to be responsible for an intermediate syndrome of neuropathy in which paralysis occurs 24 to 96 hours after exposure. The syndrome usually dissipates, if it is not fatal, 14-18 days after exposure (Baker et al. 1996; Senanayake et al. 1987).

There have been several reports on the ability of sarin to inhibit the enzyme neurotoxic esterase or neuropathy targeted esterase (NTE). NTE is a 150 kd intregral membrane protein whose function has not been fully elucidated but recent studies indicate that NTE is a lysophospholipase essential for embryonic blood vessel development and survival, and whose inhibition leads to neuronal loss (Akassoglou et al. 2004, Moser et al. 2004, Quistad 2003).

The inhibition of NTE has been reported to be responsible for the onset of organophosphate induced delayed neuropathy (OPIDN). OPIDN was responsible for the Jamaican Ginger episode described above, and usually has a time of onset of at least two weeks following exposure. (There may also be age dependence in OPIDN in some species. In chickens, OPIDN is only seen in older chickens and not in chicks.) The

pathway through which inhibition of NTE leads to OPIDN has not yet been elucidated, although it is known that neuropathy only occurs when over 70% of NTE activity is inhibited following acute exposure and 50% following repeated exposures (Ehrich et al. 2001).

OPIDN is usually not seen in acute sarin exposures at concentrations below the LD_{50} in chickens and other animals (Bucci et al. 1992, Spencer et al. 2000). Other studies have reported symptoms suggestive of OPIDN in mice exposed following repeated inhalation of sublethal doses of sarin (Husain et al. 1993). OPIDN has also not been observed in humans following severe, mild or moderate nerve agent exposures, but OPIDN theoretically could occur, particularly when prophylaxis is administered (Sidell 1997, Spencer et al. 2000).

Recent animal studies have shown no effect of pyridostigmine bromide on NTE inhibition either alone or in combination with sarin (Wilson et al. 2002). It should also be noted that subclinical neuropathy was reported 30 days after sarin exposure in Japan and a subsequent study also picked up electromyographic evidence of neuropathy six months after exposure (Morita et al. 1995, Murata et al. 1997)

Although all humans are susceptible to sarin toxicity there have been several papers that indicate polymorphisms may play a role in the development of neurotoxicity, particularly during low levels of exposure. The high-density-lipoprotein-associated enzyme paraoxonase hydrolyzes sarin and other organophosphates into essentially non-toxic products. There are two major polymorphic forms of the enzyme, the Arg192 and Gln192 isoforms. Although the Arg192 isoform hydrolyzes paraoxon more rapidly, it displays lower activity towards sarin. The dominance of the Arg192 isoform among the Japanese has been postulated to play a role in the toxicity seen in the Japanese incidents (Yamasaki 1997).

Butyrylcholinesterase levels have also been shown to correlate with protection from sarin toxicity (Raveh et al. 1993). Butyrylcholinesterase is also polymorphic. Although no studies have yet correlated butyrylcholinesterase polymorphism with toxicity, the activity of the enzyme should affect sarin toxicity (Maekawa et al. 1997).

The effect of sarin on a variety of receptors and AChE has also been studied in combination with heat and pyridostigmine bromide in attempts to mimic conditions during the Gulf War. The study with pyridostigmine showed that treatment with low levels of sarin caused an upregulation of m2 muscarinic receptors in various areas of the central nervous system (Abou-Donia et al. 2002). The study involving heat-stressed rats and low levels of sarin showed a reduction of m1 muscarinic receptors both in the presence and absence of heat and an increase in m3 receptors only in the presence of heat. The study also showed a reduction of AChE in the hippocampus with sarin and heat stress (Henderson 2002).

Another study showed modulation of nicotinic and the m2 muscarinic acetylcholine receptors following sarin exposure. An initial decrease was observed 1-3 hours post-

exposure followed by increases in both receptors at 6-20 hours. The effect on nicotinic receptors was more pronounced (Khan et al. 2000). The long-term effects of receptor modulation may be associated with memory and cognitive disorders.

Exposure to high levels of sarin causes seizures which, if untreated, can lead to substantial neuropathy. A three-phase model of nerve agent induced seizures and neuropathy has been proposed. The initial phase is a cholinergic phase that lasts from the time of exposure to approximately 5 minutes after the onset of seizures. The second phase is a progressively mixed phase involving acetylcholine and excitory amino acids such as glutamate. This phase lasts from five minutes to forty minutes after the onset of seizures. The final phase is a noncholinergic phase where extended stimulation of neurons with excitory amino acid combined with hypoxia/anoxia/ischemia leads to prolonged elevation interneuronal free Ca^{++} and resultant neurotoxicity (McDonough et al. 1997).

Exposure to high levels of organophosphates has been long known to impair the immune system (Street et al. 1975). Recently several groups have shown that subclinical exposures of sarin in rats and mice can impair both T cell responses and the bactericidal activity of macrophages. Sarin does not appear to act through the hypothalamus-pituitary-adrenal axis but through action on the autonomic nervous system. The suppression of immune function has been reported to last up to twelve months following exposure (Kassa et al. 2004, Kassa et al. 2000, Kalra et al. 2002).

Pharmacokinetics

The acute effects of sarin are believed to be primarly due to (-)-isomer of sarin. The (+)-isomer appears to be eliminated rapidly from body following administration. Animal studies indicate that (-)-sarin is rapidly distributed throughout the body, within minutes, but eliminated very slowly with a half-life of several hours (Spruit et al. 2000).

The primary metabolite of sarin is isopropyl methylphosphonic acid which was found in large amounts in the serum and urine of victims in Japan. The concentration of the metabolite and the amount of time from exposure can be used to estimate the level of exposure. These studies indicated that several of the survivors were exposed to supra-lethal levels of sarin (Noort et al. 1998, Minami et al. 1997).

Forensic Assays & Environmental Detectors

Although there are currently no real-time clinical tests for Sarin exposure, there have been a number of forensic assays developed that can confirm exposure. Most of these tests involve isolating RBC AChE and/or serum butyrylcholinesterase from blood and releasing and detecting any organophosphates that are released. One technique involves solubilizing sarin-bound AChE from tissues or blood, trypsinizing the proteins and releasing bound organophosphate with alkaline phosphatase. The organophosphate undergoes trimethylsilyl derivatization and is identified using gas chromatography (GC) (Nagao et al. 2003, Matsuda et al. 1998). Another method uses the fluoride ion to

reactivate butyrylcholinesterase and/or AChE and then quantitates by GC the arin produced. (Degenhardt et al. 2004, Jakubowski et al. 2004, Polhuijs et al. 1997).

(Diagnostic methods based upon history and observations of signs and symptoms are treated at the end of the following section on Health Effects.)

There has also been a great deal of work on the environmental detection of sarin and other nerve agents. The Department of Defense has developed several detectors to monitor air for the presence of nerve agents. The mainstay of the Army chemical detection is the M8A1 alarm which constantly samples the air for higher molecular weight molecules. The detector ionizes gases and mass filters away the low molecular ions generated from air. This is a low specificity detector and a number of other compounds can interfere (Augerson 2000, Rostker 1997). There have also been a number of other detectors developed to detect nerve agents, the NRL-SAWRHINO which uses sensors and gas chromatography has the ability to distinguish between interfering gases and nerve agents (McGill et al. 2000).

IV. HEALTH EFFECTS

Overview

The health effects of sarin, like most nerve agents, are dependent upon the route of administration, the dose received, and the speed at which treatment is given. Casualties can go from being fully functioning to comatose with severe respiratory distress in a matter of seconds following exposure. Aggressive, rapid therapy can substantially minimize adverse health effects seen in patients exposed to nerve agents (Newmark 2004). The overall experience from Iran-Iraq war, and the two Japanese attacks is that most patients who are exposed to nerve agents physically recover relatively quickly if they survive initial exposure (Newmark 2004, Ohbu et al. 1997, Murata et al. 1997).

Acute Toxicity

Acute effects seen at low concentrations include: miosis (pinpoint pupils), ocular pain, blurred or dimmed vision, tearing, rhinorrhea, broncospasm, slight dyspnea, respiratory secretions, salvation, and diaphoreses. At intermediate concentrations moderate dyspnea, nausea, vomiting, diarrhea are seen. At high concentrations convulsions, loss of consciousness, muscle fasciculations, flaccid paralysis, copious secretions, apnea and death.(Lee 2003). The LD_{50} of sarin in variety of species is listed below:

ROUTE OF EXPOSURE	SPECIES	LD_{50} (μg/kg)
Oral	Human	71-285
Oral	Rat	550-1060
Dermal	Human	1429-28,000
Dermal	Pig	115,900
Dermal	Rat	2500
Dermal	Mouse	1080
Intravenous	Human	14
Intravenous	Monkey	20
Intravenous	Pig	15
Intravenous	Rat	39-45
Subcutaneous	Rat	103-108
Intramuscular	Human	30
Intramuscular	Monkey	22
Intramuscular	Rat	108-170
Intraperitoneal	Rat	218-250

Table derived from National Research Council (US) 1999.

The acute emergency guideline levels (AEGLs) are threshold exposure limits for the general public for mild effects, serious adverse effects and lethality. For sarin the levels are expressed air concentrations applicable to exposure periods ranging from 10 min to 8

hours. The data in the table below is derived largely from rat exposure data (Hartmann 2002).

AEGL LEVEL	TIME (HOURS)	CONCENTRATION (mg/m^3)
1 (nondisabling; mild)	0.167	0.0069
1 (nondisabling; mild)	0.5	0.004
1 (nondisabling; mild)	1	0.0028
1 (nondisabling; mild)	4	0.0014
1 (nondisabling; mild)	8	0.001
2 (disabling)	0.167	0.087
2 (disabling)	0.5	0.05
2 (disabling)	1	0.035
2 (disabling)	4	0.017
2 (disabling)	8	0.013
3 (lethal)	0.167	0.38
3 (lethal)	0.5	0.19
3 (lethal)	1	0.13
3 (lethal)	4	0.07
3 (lethal)	8	0.051

Other toxic factors and exposure limits established by the US Dept. of Health and Human Services (DHHS) include: the vapor concentration X time of exposure -- in which 50% lethality is seen for humans (LCt_{50}) at 100 mg/m^3/min. The no death dose equals 10 mg/m^3/min; the no neuromuscular (NNM) effect dose equals 4 mg/m^3/min; the concentration which induces miosis in 50% of victims (ECt_{50} (miosis)) equals 2-4 mg/m^3/min. The no observable effect level (NOEL) equals 0.5 mg/m^3/min; the maximal single concentration for 1 hour equals 0.001 mg/m^3; the maximal single concentration for 8 hours equals 0.0003 mg/m^3; the safety factor of 0.1 is used for the general population and the limit levels are 0.0001 mg/m^3 for 1 hour, 0.00003 mg/m^3 for 8 hours and 0.000003 mg/m^3 for 72 hours (Moore 1998).

Developmental and Reproductive Toxicity

There has been no evidence in humans of reproductive or developmental toxicity. There is no evidence of developmental toxicity in rats dosed up to 380µg/kg/d or in rabbits dosed 15µg/kg/d and who were exposed over several days of gestation that produced maternal toxicity (Laborde et al. 1996). In other studies rats were exposed for 1 week to 1 year and then mated with either exposed or unexposed rats. There was no evidence of sarin related adverse effects with respect to reproductive performance, fetal toxicity, and teratogenesis (National Research Council (US) 1999).

Carcinogenicity

There is no evidence of carcinogenicity in humans. In chronic inhalation studies in mice, rats and dogs, sarin did not appear to be carcinogenic. Pulmonary tumors were observed

in strain A mice after 3/19 and 3/20 animals after 52 weeks of exposure to 0.001 and 0.0001 mg/m^3, respectively, while controls showed no tumors. The results were not considered significant, however, since strain A mice are very susceptible to tumors and rates at six months were 5/19, 6/18, and 9/29 for mice dosed at 0.001 and 0.0001 mg/m^3 and controls, respectively (National Research Council (US) 1999, Munro et al. 1994).

Genotoxicity

There is little information available regarding the human genotoxicity of sarin. In bioassays using bacteria and mammalian cell cultures with and without metabolic activation sarin did not show any evidence of genotoxic or mutagenic activity. There was no increase in mutations using the Ames test. There was also no increase in mutations when mouse L5718 cells were tested at concentrations of 200µg/mL (National Research Council (US) 1999). There was also no evidence of sister chromatid exchanges (SCE) in Chinese hamster ovary cells at concentrations up to 1.4 mM (Nasr et al. 1988). Several studies of the victims of the Tokyo subway attack, however, indicated that the SCE of lymphocytes was higher in persons exposed to sarin. These also revealed a positive correlation between the extent of serum cholinesterase inhibition and the level of SCE. These studies indicate that sarin exposure may lead to SCE and the effect appeared to last up to three years after exposure. One paper indicates that the synthetic by-products diisopropylmethylphosphonate (DIMP) or diethylmethylphosphonate (DEMP) may be responsible for the increase in SCE (Li et al. 2004, Li et al. 2000, Minami et al. 1998).

Immunotoxicity

Although there is no direct evidence of human immunotoxicity, there are several animal studies that indicate there is a potential of some immunotoxicity or immunomodulatory effects upon sarin exposure. Experiments in rats has indicated that exposure at 0.75 LD_{50} significantly reduced T-cell mediated immune reaction. The reduced activity was attributed to be due the inhibition of a variety of esterases (Zabrodskii et al. 2003). Another study involving BALB/c mice dosed at subclinical levels showed a substantial increase in NK cell and macrophage activity, and a substantial decrease in CD4 T-cell activity. The studies also showed that a single exposure has the same effect as multiple exposures. Studies in rats have also shown a decrease in T-cell activity (Kassa et al. 2004a, Kassa et al. 2004b, Kalra et al. 2002). Other studies have shown significant reductions in NK cell, cytotoxic T-cell activity by the DIMP and DEMP by-products. The sarin by-products presumably work through the inhibition of granzyme function (Li et al. 2000, Li et al. 2002).

Eye and Visual Effects

Miosis, as noted above, is characteristic of sarin exposure and often the first sign. It usually occurs within seconds or minutes of exposure and can last up to 9 weeks. Dim vision, which consists of a reduction of light entering the eye, is often the result of miosis. Blurred vision and eye pain can also accompany sarin exposure. Intraocular pressure and color do not appear to be affected by sarin (Sidell 1997, Rengstorff 1985).

Otic Effects

There is very little data on the effect of sarin on hearing. Animal studies indicate that sarin cause increased acoustic startle in rats. The effect was not seen in rats also treated with pyridostigmine bromide (Scremin et al. 2003).

Pulmonary Effects

Rhinorrhea is typically seen shortly after sarin exposure. The rhinorrhea can be quite intense and has been described as much worse from that seen with any cold or hay fever. These secretions make it difficult to attempt artificial respiration if needed. Respiratory effects are also very common and occur rapidly after exposure. The effects are caused by chemical irritation; and the effect of acetylcholine on nicotinic, muscarinic, and central nervous neurons. Tightness in the chest is a common symptom after exposure to small amounts of sarin and usually dissipates within hours of exposure. As the amount of exposure increases dyspnea and pulmonary distress increase and often someone severely poisoned will go into respiratory failure and die. There is not any data that indicates respiratory effects persist long after exposure (Sidell 1997**,** Niven et al. 2004).

Cardiac Effects

Bradycardia is frequently seen following moderate or high level sarin exposure. Bradycardia may be caused from stimulation of the atrial-ventricular node through the vagus nerve (Sidell 1997). Although bradycardia is frequently resolved following treatment, there have been reports of persistent arrhythmias following exposure. In cases of severe poisoning cardiomyopathy may also be seen (Okudera 2002). Studies in rats have shown cardiomyopathy following high doses of sarin (Singer et al. 1987). Other rat studies have revealed that acute dosages of sarin induce QT prolongation and cardiac lesions (Abraham et al. 2001**).** Electocardiograms showed that decreases in the graphic R-R interval variability (CV_{RR}) as well as the C-C_{LF}, C-C_{HF} taken six months after exposure correlated with serum AChE levels (Murata et al. 1997).

Skeletal Muscle Effects

Neuromuscular effects have been studied since nerve agents were discovered. Acetylchloline is a primary neurotransmitter at the neuromuscular junction. Increased acetylcholine initially leads to stimulation, followed by fatigue and paralysis of muscle fibers, muscle and muscle groups (Sidell 1997). In the Tokyo attack, asthenia or muscle weakness was seen in most patients upon admission to the hospital (Murata et al 1997). Following liquid exposure, muscle fasciculations at the site of exposure are often seen following excessive sweating (Lee 2003). Long-term shoulder stiffness has also been associated with sarin exposure although it is not clear the incidence is greater than a control population (Nakajima 1999). Myopathy has also been seen in rats in the absence of treatment following a moderate dose of sarin (Gupta et al. 1992).

Nervous System Effects

There exists a good deal of data on the neurotoxicity of sarin in humans. The role of sarin in inducing convulsions and the resultant neuropathology was described above (McDonough et al. 1997). Reports from the Japanese sarin terrorist incidents reveal that most patients who were exposed and survived have not suffered from persistent neurological disorders. There have been exceptions, however. One is a patient from Matsumoto who suffered from akinetic mutism for at least two years following exposure. Studies in rats have shown that there is wide variability in neurotoxicity following repeated sublethal doses of sarin. These studies showed that there is a lack of tolerance with repeated doses and a cumulative effect on toxicity (Fernado et al. 1985).

Although the mechanism of action has not been described, headaches are a very common symptom of sarin exposure (Ohbu et al. 1997, Murata et al.). Loss of memory is also common and there has even been one case of amnesia following exposure (Hatta et al. 1996, Hood 1997). Long term changes in electroencephalograms (EEG) in workers following accidental sarin exposure have been observed. The study revealed increases in beta and delta activity, and a slowing in theta activity. The study also showed increased REM sleep. These findings have been somewhat controversial and it is not known if abnormal EEG is actually characteristic any clinically relevant syndromes. (Duffy et al. 1979; National Research Council (US) 1999).

Discussion regarding possible psychogenic effects of sarin exposure is treated in the full section on Psychogenic Effects.

Diagnosis of Fact & Extent of Intoxication

The initial diagnosis of sarin exposure is based on signs, symptoms and historical factors. The first step in the diagnosis is to confirm presence of both nicotinic and muscarinic effects. A convenient mnemonic for the signs and symptoms of nerve agent poisoning is dumbbels, which stands for the following:

Diaphoresis, diarrhea
Urination
Miosis
Bradycardia
Bronchospasm and bronchorrhea
Emesis
Lacrimation, rhinorrhea, and salivation
Seizures, muscle fasciculation, weakness (PIER 2004)

Symptoms depend on the site and extent of exposure. Following dermal contact symptoms can be delayed for up to18 hours, symptoms from inhalation can occur within seconds of exposure. Miosis is commonly the first symptom seen following vapor exposure due to the relatively low threshold value of 0.5 mg-min/m^3 and to the volatility

of sarin. Sarin is more volatile that any of the other nerve agents so there is usually always vapor exposure whenever sarin is released. Percutaneous absorption of liquid sarin also occurs readily and typically leads to localized sweating, followed by muscular fasciculations and weakness (Lee 2003, National Research Council 1997). Useful markers of nerve agent exposure include serum butyrylcholinesterase and red blood cell (RBC) AChE activity. Both of these enzymes react with sarin; significantly reduced levels are indicative of nerve agent exposure, although it does not absolutely correlate with toxicity (Suziki et al. 1997). Analysis of patients from the Tokyo subway event however indicated that miosis could be a better indicator of potential systemic toxicity than red blood cell AChE levels (Nozaki et al. 1997). In addition to the effects on AChE levels, high levels sarin exposure has been shown to reduce serum triglycerides, potassium and chloride and to cause increases in serum creatine phosphokinase (CPK), leukocytes and ketones in urine (Morita et al. 1995, Minami et al. 1998).

In many countries other than the US, the Peradeniya Organophosphorous Poisoning (POP) scale is used to assess the extent of organophosphate poisoning. The values in the scale are described below:

PARAMETER	FINDING	SCORE
Miosis	Pupil size > 2mm	0
	Pupil size ~ 2mm	1
	Pupils Pinpoint	2
Fasciculation	None	0
	Present not generalized or continuous	1
	Generalized and continuous	2
Respiration	Respiration Rate (RR) =20 min^{-1}	0
	RR > 20 min^{-1}	1
	RR > 20 min^{-1} with central cyanosis	2
Bradycardia	Heart rate (HR)> 60 min^{-1}	0
	HR 41-60	1
	HR < 41	2
Level of Consciousness	Conscious and rational	0
	Impaired, responds to verbal commands	1
	Impaired, no response to verbal commands	2
Convulsions	Absent	0
	Present	1

The total possible score is 11; the higher the score, the worse the patient prognosis. Death has not been seen in patients who have a score of three or less (Wiener et al. 2004).

V. PSYCHOGENIC EFFECTS

Because of the nature of nerve agents and the terror they create, psychogenic effects are very common following their use or perceived use. In the Iran-Iraq war, soldiers would frequently inject themselves with atropine and insist they had been exposed to nerve agents, when all evidence indicated that they had not been exposed (Newmark 2004a). In the Tokyo attacks, over 5000 persons reported to hospitals to be treated while, by the best estimate, only about 1000 people were actually exposed (WHO 2001). Because of the high prevalence of psychogenic effects and the need for immediate treatment upon actual exposure, it is important to be able to distinguish between psychogenic events and actual exposure.

Post-traumatic stress disorder (PTSD) was seen in a number of sarin victims in Japan. Several studies have shown persistent decreases in serum cholinesterase activity in patients with PTSD. The studies also showed no correlation in serum cholinesterase activity taken right after exposure and the development of PTSD six months later (Tochigi et al. 2002, Nakajimi et al. 1999**,** Murata et al. 1997).

Typical symptoms seen in PTSD include fatigue, asthenia, insomnia, blurred vision, general anxiety. MRI studies have shown a negatively correlation with the gray-matter volume in the left anterior cingulate cortex (ACC). The ACC is believed to be involved in attention, emotional regulation and conditioned fear -- all factors recognized in the development of PTSD (Yamasue et al. 2003).

A survey study of volunteer participants in the 1955-1975 Edgewood military testing of antichoinesterase agents found that those who had undergone the testing subsequently experienced greater sleep disturbance than those who had undergone no chemical tests. Further, volunteers who reported exposure to civilian or military chemical agents independent of participation in the Edgewood program reported a number of adverse neurological and psychological effects, beyond that reported by those who experienced only experimental exposure. Self-reported experiences of exposure which are subject to recall bias appear to have induced greater psychological health effects consequences than actual experimental agent exposure (Page 2003).

A survey of general effects of perceived exposure to chemical and biological warfare agents is contained in the supplement under this contract "Psychogenic Effects of Perceived Exposure to Biochemical Warfare Agents."

VI. TREATMENT & PREVENTION

There are essentially five components of treatment for sarin exposure. The first component is prophylaxis. This is typically accomplished by the administration of pyridostigmine, a carbamate that reacts reversibly with AchE protecting the enzyme from inactivation. Studies have shown, however, that physostigmine is more effective than pyridostigmine in reducing the toxicity of sarin. A phosphotriesterase enzyme has been shown more effective than any of the carbamates (Tuovinen et al. 1999). Human butrylcholinestrase has also been shown to be effective in reducing toxicity by essentially acting as a dead-end target and inactivating enzyme for nerve agents (Raveh et al. 1993).

The second component of treatment is decontamination and evacuation. Evacuation and extensive decontamination not only protects the victim from further exposure but also protects health care workers who may come in contact with the victim. Decontamination consists of removing all clothes and extensively washing the patient, particularly the patient's hair. It is important to provide health care in an area that is free from nerve agents (CDC 2004)

The third component of treatment is the use of anticholinergic agents to block the effect of increased acetylcholine at synapses. Atropine is commonly used for this purpose. It demonstrates nanomolar inhibition activity against all three subtypes of muscarinic receptors. One paper has postulated that selective inhibition of m2 and m3 receptors may be more effective in the treatment of sarin exposure (Trovero 1998). There are currently no agents routinely used to block the acetylcholine activity at nicotinic receptors.

The fourth component is the use of oximes to regenerate AchE enzymes. There have been a number of oximes that are capable of regenerating AchE enzyme. Obidoxime and pralidoxime are the only oximes that have been approved for this use. Pralidoxime is the agent of choice in the US military, while obidoxime is used in Europe and other part of the world (Newmark 2004a, Newmark 2004b, Kassa 2002). Oximes, to be effective, have to be used prior to aging; the half-life of aging of sarin on the AchE enzyme is 5 hours (CDC 2004).

The fifth component of treatment is the use of anticonvulsants. Diazepam has been the mainstay of anticonvulsant therapy for nerve agent poisoning. Although diazepam is generally thought to act through gamma-aminobutyric acid A (GABAA) receptors, its effects on nerve agent poisoning may work through other mechanisms. Diazepam should only be given for high dose exposure when a patient is having seizures. In hospital setting it should always be administered IV because availability by IM is usually low (Marrs 2003, Tuovinen 2004).

In addition to the treatments mentioned above there has also been interest in using adenosine agonists such as N6-cyclopentyladenosine (CPA) to attempt to decrease the amount of acetylcholine released at synapses. CPA has shown promise in reducing the potential cardiovascular toxicity following sarin exposure (Joosin et al. 2004).

The Iranians have the most extensive experience in treating nerve gas exposure. Unfortunately, there is very little published work on the strategies used and the lessons learned during the Iran-Iraq war. A recent paper chronicles the work of Syed Abbas Foroutan, an Iranian physician primarily responsible for treatment strategies throughout the war. The Iranian army, like the US military, realized that forward treatment is essential for survival and reducing morbidity. To accomplish this, the Iranian military distributed to all soldiers three autoinjecters containing 2 mg of atropine. Atropine is an anticholinergic agent that has rapid IM uptake and dispersion and binds to muscarinic cholinergic receptors. Rapid treatment can restore the normal function of muscarinic receptors. Foroutan, unlike the US military and NATO, relied primarly on atropine to treat most of the victims. His failure to use oximes was partly due to the lack of availability and also to his belief that rapid atropinization was essential for treatment (Newmark 2004a).

Foroutan used extensive amounts of atropine, sometimes up to 200 mg. He used the ease of breathing and the drying of respiratory secretions as the standard to which treatment doses should be titrated. Like NATO, he thought that miosis should not be used as an endpoint to determine if enough atropine has been used. As mentioned above, Foroutan disagreed with NATO guidelines on the rate of atropinization. Frontline Iranian soldiers did not have either oximes or diazepam autoinjecters to provide treatment at the site of exposure. Foroutan used obidoxime instead of pralidoxime based on the mistaken notion that obidoxime had a longer half-life. Oximes regenerate AchE but must be used prior to sarin undergoing aging. The lack of oximes at the frontline may have undermined the effectiveness of this therapy. Foroutan also used diazepam, not only to control seizures but also but also as a muscle relaxant. The Iranians did not appear to use any prophylactic agents to minimize the effect of sarin exposure (Newmark 2004a).

VII. SECONDARY SOURCE COMMENT

The literature on sarin is huge. About 700 references can be found in PubMed alone on a search for sarin. A Scirus search returns over 1700 journal references.

Despite the great amount of literature, perhaps the most notable gap on sarin is the relative absence of data (a fact alluded to throughout this report) from sarin exposures in the Iran-Iraq war and the former Soviet Union.

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. This bibliography contains supplemental material on the subject in addition to references that are specifically cited in the text above.}

Abou-Donia et al. 2002. Sensorimotor deficit and cholinergic changes following coexposure with pyridostigmine bromide and sarin in rats. *Toxicol Sci.* 66(1):148-58. A myriad of neurological symptoms including muscle and joint pain, ataxia, chronic fatigue, headache, and difficulty in concentration have been reported by Persian Gulf War (PGW) veterans. A large number of these veterans were prophylactically treated with pyridostigmine bromide (PB) and possibly exposed to sarin. In the present study we investigated the effects of PB and sarin, alone and in combination, on sensorimotor performance and the central cholinergic system of rats. Male Sprague-Dawley rats were treated with PB (1.3 mg/kg, 15 daily doses, oral) and sarin (50, 75, 90, and 100 microg/kg, single im dose on day 15), alone and in combination. The animals were evaluated for postural reflexes, limb placing, orienting to vibrissae touch, incline plane performance, beam-walk time, and forepaw grip time 7 and 15 days following treatment with sarin. Treatment with either PB or sarin alone resulted in significant sensorimotor impairments. Coexposure to sarin and PB resulted in significant sensorimotor deficits that worsened over time. By 15 days following sarin treatment, plasma butyrylcholinesterase (BChE) activity returned to normal levels in the animals treated with sarin alone, whereas in the animals exposed to PB or PB plus sarin, there was an increase in the enzyme activity. Cortical acetylcholinesterase (AChE) activity remained inhibited in the animals treated with sarin alone and in combination with PB. Muscarinic acetylcholine receptor (m2 mAChR) ligand binding with [(3)H]AFDX-384 in cortex and brain stem showed significant increases (approximately 120-130% of control) following coexposure to PB and sarin at higher doses. To evaluate the potential of PB for augmentation or inhibition of the toxicity induced by acute sarin exposure, the animals were exposed to either 10 or 100 microg/kg sarin (single im injection) with or without pretreatment with PB, and sacrificed 3 h after treatment with sarin. Pretreatment with PB offered slight protection in the plasma as well as brain regional enzyme activities. Pretreatment with PB did not have any effect on sarin-inhibited brain regional AChE activity following treatment with 100 microg/kg sarin. These results show that prophylactic treatment with PB offers some degree of protection in peripheral cholinesterase. Furthermore, these results show that treatment with either sarin or PB alone resulted in sensorimotor impairments, while coexposure to high doses of sarin with PB caused an exacerbated deficit.

Abraham et al. 2001. QTc prolongation and cardiac lesions following acute organophosphate poisoning in rats. *Proc West Pharmacol Soc*. 44:185-6.

Sarin and soman induced the following similar effects: prolongation of QT interval duration, cardiac lesions and immediate and statistically significant decrease in body weight. However, animals exposed to soman remained underweight and suffered delayed death. Thus, as sarin produced both cardiac lesions and QT prolongation, without exhibiting late death, it is unlikely that the late death observed in soman-poisoned rats are attributable to QT prolongation and the occurrence of life-threatening arrhythmias. It is postulated that low body weight may precipitate late mortality in soman-exposed rats. It is well documented that QT prolongation in the rat is explained in terms of blockade of the Ito potassium channels and the Na+/Ca+2 exchanger. Soman and sarin may exert their effect on QT interval duration through non-specific action on these sites. As drug-induced QT prolongation in man is mediated by blockade of Ikr potassium channels, the data presented in this study may not predict late death in humans in cases of organophosphate intoxication.

Akassoglou et al. 2004, Brain-specific deletion of neuropathy target esterase/swisscheese results in neurodegeneration**.** *Proc Natl Acad Sci USA*. 101(14):5075-80.
Neuropathy target esterase (NTE) is a neuronal membrane protein originally identified for its property to be modified by organo-phosphates (OPs), which in humans cause neuropathy characterized by axonal degeneration. Drosophila mutants for the homolog gene of NTE, swisscheese (sws), indicated a possible involvement of sws in the regulation of axon-glial cell interaction during glial wrapping. However, the role of NTE/sws in mammalian brain pathophysiology remains unknown. To investigate NTE function in vivo, we used the cre/loxP site-specific recombination strategy to generate mice with a specific deletion of NTE in neuronal tissues. Here we show that loss of NTE leads to prominent neuronal pathology in the hippocampus and thalamus and also defects in the cerebellum. Absence of NTE resulted in disruption of the endoplasmic reticulum, vacuolation of nerve cell bodies, and abnormal reticular aggregates. Thus, these results identify a physiological role for NTE in the nervous system and indicate that a loss-of-function mechanism may contribute to neurodegenerative diseases characterized by vacuolation and neuronal loss.

Augerson 2000. Nerve agents. Chapter 5 In *A Review of the Scientific Literature as it Pertains to Gulf War Illnesses Volume 5: Chemical and Biological Warfare Agents***.** Rand Corporation. Available at:
http://www.rand.org/publications/MR/MR1018.5/MR1018.5.chap5.html

Baker et al. 1996. Single fibre electromyographic changes in man after organophosphate exposure. *Hum Exp Toxicol.* 15(5):369-75.
1. Neuromuscular (NM) changes resulting from organophosphate exposure are known to be complex. After severe acute poisoning recovery from initial depolarisation paralysis may be followed in a limited number of cases by onset of a non-depolarisation paralysis (the Intermediate Syndrome). It is not clear whether this block arises subclinically in all cases of poisoning as a sequel to the initial depolarisation. 2. Single fibre electromyography (SFEMG) is a sensitive clinical neurophysiological technique allowing detection of subclinical changes at the neuromuscular junction. In the study reported it

has been used to examine changes in NM transmission in the forearm of fit volunteers exposed to a low level of sarin (isopropyl methyl phosphonofluoridate). 3. Small changes in SFEMG were seen at three hours and three days after an exposure sufficient to cause a reduction in red cell acetyl cholinesterase to 60% of normal. The SFEMG changes were not accompanied by any clinical neuromuscular symptoms or signs and returned to normal 2 years after exposure. 4. The results indicate that there are reversible subclinical changes compatible with the development of non-depolarising NM block without frank clinical expression. In the small population examined there were individual variations in response which may reflect differences in safety margin at the neuromuscular junction.

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Benschop et al. 2001. Toxicokinetics of Nerve Agents. In *Chemical Warfare Agents: Toxicity at Low Levels* (SM Somani, JA Romano Jr. Eds) Chapter 2, CRC Press Boca Raton Fl.

Bismuth et al. 2004. Chemical weapons: documented use and compounds on the horizon. *Toxicol Lett.* 149(1-3):11-8.
Man's inhumanity to man is expressed through a plethora of tools of modern warfare and terror. The use of chemical and biological weapons with the goals of assault, demoralisation and lethality has been documented in recent history, both on the battlefield and in urban terror against civilians. A general review of a few of the currently employed chemical weapons and biological toxins, along with a look at potential chemical weapons and tools of counter-terrorism, follows. While these weapons are fearsome elements, the dangers should be viewed in the context of the widespread availability and efficacy of conventional weapons.

Bucci et al. 1992. Toxicity Studies on Agents GB and GD (Phase II): Delayed Neuropathy Study of Sarin, Type II, in SPF White Leghorn Chickens. National Center for Toxicological Research Final Report; AD-A257183.

Burchfiel et al. 1982. Organophosphate neurotoxicity: chronic effects of sarin on the electroencephalogram of monkey and man. *Neurobehav Toxicol Teratol*. 4(6):767-78.
The neurotoxic effects of the organophosphate sarin (107448), on the electroencephalograms (EEGs) on monkeys and humans were investigated. In the animal study, electrodes were permanently implanted in rhesus-monkeys for chronic EEG recording. Animals received intravenous injections of either a single large dose of 5 micrograms per kilogram (microg/kg) sarin or multiple small doses (ten injections) of 1microg/kg sarin intramuscularly at 1 week intervals. In both cases EEG recordings were taken 24 hours after administration. One year after exposure three additional recordings

were performed with animals awake in light, awake in darkness, and in drowsy states. The human study consisted of 77 industrial workers with a documented history of at least one exposure to sarin. Each subject had two EEG recording sessions. EEG data was obtained either under clinical laboratory or all night sleep conditions. Five recording states were used for spectral EEG analysis: eyes open, eyes closed, drowsy, hyperventilation, and post hyperventilation. In monkeys, there was a persistent increase in beta activity in the temporal lobe EEG of animals treated with large and small doses of sarin. The beta increase was present at 24 hours after drug administration and persisted 1 year later for most animals. At 24 hours post drug, significant changes in other frequency bands were observed, but EEG alterations disappeared within a few days or weeks. In the human study, the major differences in spectral analysis of EEGs were increases in beta activity in the sarin exposed population. For sleep EEG records, the exposed population had significantly increased rapid eye movement sleep. Univariate and multivariate spectral analyses resulted in significant differences between comparisons and exposed groups. The authors conclude that in both humans and primates the major long term effect of sarin on EEGs is an enhancement of higher frequency (beta) activity.

CDC 2004. NIOSH Emergency Response Card:Sarin. Available at: http://www.bt.cdc.gov/agent/sarin/erc107-44-8.asp.

Chebabo et al. 1999. The organophosphate sarin, at low concentrations, inhibits the evoked release of GABA in rat hippocampal slices. *Neurotoxicology*. 20(6):871-82. In the present study, the whole-cell mode of the patch-clamp technique was applied to neurons of the CA1 pyramidal layer of rat hippocampal slices to investigate the effects of the organophosphate (OP) sarin on field stimulation-evoked and on tetrodotoxin (TTX)-insensitive postsynaptic currents (PSCs) mediated by activation of type A gamma-aminobutyric acid (GABA) receptors or AMPA-type glutamate receptors. At 0.3-1 nM, sarin reduced the amplitude of GABA-mediated PSCs and had no effect on the amplitude of glutamatergic PSCs evoked by field stimulation of neurons synaptically connected to the neuron under study. The effect of sarin on evoked GABAergic PSCs was unrelated to cholinesterase inhibition, was partially reversed upon washing of the neurons with sarin-free external solution, and was mediated by a direct interaction of the OP with muscarinic acetylcholine receptors present on presynaptic GABAergic neurons. Sarin had no effect on the amplitude or kinetics of GABA- or glutamate-mediated miniature postsynaptic currents (MPSCs) recorded in the presence of the Na+-channel blocker TTX (300 nM), indicating that the OP does not interact with GABA(A) or glutamate receptors. Further, sarin did not alter the frequency of GABAergic or glutamatergic MPSCs, a finding that led to the conclusion that this OP does not affect the TTX-insensitive release of neurotransmitters. A selective reduction by sarin of the action potential-dependent release of GABA in the hippocampus can account for the occurrence of seizures in intoxicated subjects.

Chi et al. 1995. Action of organophosphate anticholinesterases on the three conformational states of nicotinic receptor. *Adv Exp Med Biol.* 363:65-73. Organophosphate and other ligands were examined for binding on the membrane-bond nicotinic receptor at three conformational states. Soman (pinacolyl

methylphosphonofluoridate), sarin (isopropyl methylphosphonofluoridate, tabun (ethyl N-dimethylphosphoramidocyanidate) and phencyclidine did not show any effect on the binding of [25I]alpha-cobrotoxin to the nicotinic receptor. However, VX, O-ethyl-S-(2-diisopropylaminoethyl) methylphosphonothiolate, at concentrations higher than 10 umol/L exhibited profound inhibition on the equilibrium binding rates in a concentration-dependent manner. Agonist nicotine and antagonist d-tubocurarine also showed significant inhibitions.

Costa et al. 1999. The role of paraoxonase (PON1) in the detoxication of organophosphates and its human polymorphism. *Chem Biol Interact.* 119-120:429-38. In human populations, serum paraoxonase (PON1) exhibits a substrate dependent polymorphism. The Arg192 isoform hydrolyzes paraoxon rapidly but diazoxon, soman and especially sarin slowly. On the other hand, the Gln192 isoform hydrolyzes paraoxon slowly, but diazoxon, soman and sarin more rapidly than the Arg192 isoform. Our experiments with a mouse model system have convincingly shown that PON1 plays a major role in the detoxication of organophosphate (OP) compounds processed through the P450/PON1 pathway. Recent studies have also shown that PON1 plays an important role in the metabolism of oxidized lipid compounds. Currently, there is an effort underway to identify genes and polymorphisms that play an important role in 'environmental susceptibility'. The PON1 polymorphism has been cited as a prime example of such a genetic polymorphism. The advent of the polymerase chain reaction (PCR) for DNA amplification with improvements, modifications and automation has provided a very convenient way to do individual genotyping. It is tempting to set up large scale PCR analyses of populations to determine individuals at risk for environmental exposures affected by the PON1 polymorphism. In fact, a number of such studies have already been carried out in examining the relationship of the PON1 polymorphism to vascular disease. We advocate the use of a high throughput two-dimensional enzyme assay that provides both PON1 genotype and phenotype (PON1 status). The high level of variation of gene expression within each genetic class in humans, together with our animal model studies indicate that it is very important to determine PON status as opposed to PON1 genotype alone. Experiments in rats and mice have shown that injection of PON1 purified from rabbit serum by the i.v., i.p. or i.m. route, significantly increases PON1 activities in rodents' plasma. Under these conditions, the acute toxicity (assessed by the degree of acetylcholinesterase inhibition) of paraoxon and chlorpyrifos oxon is significantly decreased, compared to control animals. Protection is maximal when PON1 is administered before the OPs, but still occurs when PON1 is utilized as a post-exposure treatment. Furthermore, protection by PON1 is also provided toward the parent compound chlorpyrifos. Pon1-knockout mice display a much greater sensitivity to chlorpyrifos oxon toxicity than wild mice. However, the acute toxicity of guthion, which is not a substrate for PON1, does not differ between knockout and wild mice. These observations underline the importance of considering both genetic variability of enzyme isoform as well as enzyme level (PON1 status) and the developmental time course of appearance of PON1 in developing risk assessment models.

CWC 2004A. Background of Chemical Disarmament.

In the time between April 1997 and the present day, the OPCW has grown into a major international organisation in the field of arms control and disarmament. It has grown faster than any other global disarmament regime in history, from the original 87 States Parties to 162 as of 27 April 2004. The CWC commits States Parties to destroy all stockpiles of chemical weapons by 2007. So far, the OPCW has overseen the destruction of nearly 12 percent of the world's stockpile.
The OPCW has a full-time staff of around 500 international civil servants and an approved budget for 2004 of 73,153.390 million euros a year. As of October 2000, the OPCW and the UN entered into a formal agreement for the exchange of information, resources, and personnel. The CWC regime now covers 95 percent of the world's population, 92 percent of the world's landmass, and 98 per cent of its chemical industry. Never before in history have the countries of the world been so close to the destruction of an entire category of weapons of mass destruction. It is only with the complete commitment of all its States Parties to the cause of multilateral disarmament that the OPCW will be able to complete its task and its fundamental goal: a chemical weapons-free world.
Available at: http://www.opcw.org/html/intro/chemdisarm_frameset.html

CWC 2004B. Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on Their Destruction. Available at: http://www.opcw.org/docs/cwc_eng.pdf

Council on Foreign Relations 2004. Terrorism: Questions & Answers: Sarin. Available at: http://cfrterrorism.org/weapons/sarin.html

Degenhardt et al. 2004. Improvements of the fluoride reactivation method for the verification of nerve agent exposure. *J Anal Toxicol.* 28(5):364-71.
One of the most appropriate biomarkers for the verification of organophosphorus nerve agent exposure is the conjugate of the nerve agent to butyrylcholinesterase (BuChE). The phosphyl moiety of the nerve agent can be released from the BuChE enzyme by incubation with fluoride ions, after which the resulting organophosphonofluoridate can be analyzed with gas chromatography-mass spectrometry (GC-MS). This paper describes recent improvements of the fluoride-induced reactivation in human plasma or serum samples by enhancing the sample preparation with new solid-phase extraction cartridges and the MS analysis with large volume injections. Analysis is performed with thermal desorption GC with either mass selective detection with ammonia chemical ionization or high-resolution MS with electron impact ionization. The organophosphorus chemical warfare agents analyzed in this study are O-ethyl S-2-diisopropylaminoethyl methylphosphonothiolate, ethyl methylphosphonofluoridate, isopropyl methylphosphonofluoridate (sarin, GB), O-ethyl N,N-dimethylphosphoramidocyanidate, ethyl N,N-dimethylphosphoramidofluoridate, and cyclohexyl methylphosphonfluoridate. Detection limits of approximately 10 pg/mL plasma were achieved for all analytes, which corresponds to 0.09% inhibition with GB on a sample with normal BuChE levels.

DeploymentLink 2003. Project 112 Tests Available at: http://deploymentlink.osd.mil/current_issues/shad/shad_chart/shad_chart_8_3.shtml

Contains a table of all the tests involved in Project 112 with links to fact sheets on the tests.

Duffy et al. 1979. Long-term effects of an organophosphate upon the human electroencephalogram. *Toxicol. Appl. Pharmacol.* 47(1):161-176.
The brain electrical activity of workers exposed to the organophosphate compound (OP), sarin, was compared to that of control subjects. Exposed workers had a history of one or more documented accidental exposures to toxic levels of sarin. However, no exposed subject had exposure within 1 year of his examination. The comparison included standard clinical electroencephalograms (EEGs), computer-derived EEG spectral analysis, and standard overnight sleep EEGs. It was not possible to diagnose subjects individually by expert visual inspection of their EEGs. However, statistically significant between-group differences for both the visually inspected and computer-derived data were reported by both univariate and multivariate statistical methods. Different EEG changes revealed by visual inspection and computer-derived spectral analysis appear to reflect the differing sensitivities of these two analytic techniques. Statistically significant group differences included increased beta activity, increased delta and theta slowing, decreased alpha activity, and increased amounts of rapid eye movement sleep in the exposed population. It is suggested the the above findings represent an unexpected persistence of known short-term OP actions. It is also suggested that these results, when taken along with the reported long-term behavioral effects of OP exposure, provide parallel evidence that OP exposure can produce long-term changes in brain function.

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Federation of American Scientists 2004. Chemical Weapons Programs: History. Available at: http://www.fas.org/nuke/guide/iraq/cw/program.htm

Fernando et al. 1985. Variability of neurotoxicity of and lack of tolerance to the anticholinesterases soman and sarin in the rat. *Res Commun Chem Pathol Pharmacol.* 48(3):415-30.
The neurotoxicity and lethality of Soman and Sarin, after single and repeated treatment at 50-60% of their LD50 doses in rats were investigated. Single treatment with Soman (100 micrograms/kg) and Sarin (120 micrograms/kg) produced severe tremors, convulsions and hypothermia, in some rats only, while the others did not show toxicity, i.e. an all or none effect. Soman and Sarin (100-120 micrograms/kg) caused, respectively, 89-93% and 26-48% inhibition of AChE at 6 hr and 56-68% and 17-39% inhibition at 24 h after single injections. Repeated treatment with Soman (90 micrograms/kg) and Sarin (100 micrograms/kg) at 4 day intervals caused variable incidences of neurotoxicity and increasing mortalities, and after ten injections the survival rates were 31 and 54%, and AChE inhibition was 86 and 75%, respectively. It is suggested, that the variable neurotoxicity of and the low tolerance to of these compounds are partly related to peripheral dispositional mechanisms. Furthermore, the profile of toxicity of these anticholinesterase agents should be differentiated from, but not generalized with, that of the other anticholinesterase organophosphates.

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Gosden et al. 2002. Lesson of Iraq's Mass Murder. *Washington Post,* June 2, 2002 B-07. Available at: http://www.deconsolutions.com/pdf_Files/Post06022002.pdf

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. Copyright 2002 Elsevier Science (USA)

Hatta et al. 1996.. Amnesia from sarin poisoning**.** *Lancet* 347(9011):1343.

Henderson et al. 2002. Response of rats to low levels of sarin. *Toxicol Appl Pharmacol.* 184(2):67-76.
The purpose of this study was to determine whether exposure to levels of sarin causing no overt clinical signs would cause more subtle, adverse health effects that persisted after the exposure ended. Inhalation exposures of male Fischer 344 rats to 0, 0.2, or 0.4 mg/m(3) of sarin for 1 h/day for 1, 5, or 10 days under normal (25 degrees C) and heat-

stressed (32 degrees C) conditions were completed and observations were made at 1 day and 1 month after the exposures. The sarin exposures had no observed effects on body weight, respiration rate, and minute volume during exposure nor in body temperature and activity during the 30-day recovery period. There was no evidence of cellular changes in brain determined by routine histopathology nor of any increase in apoptosis. Brain mRNA for interleukin (IL)-1beta, tumor necrosis factor-alpha, and IL-6 was increased in a dose-dependent manner. Autoradiographic studies demonstrated that M1 cholinergic receptor site densities were unchanged at 1 day after repeated exposures with or without heat stress. At 30 days, there was a decrease in M1 receptors in the olfactory tubercle (with and without heat), and, with heat stress, M1 sites also decreased in a dose-dependent manner in the frontal cortex, anterior olfactory nucleus, and hippocampus. M3 receptor sites were not affected by sarin exposure alone. In the presence of heat stress, there was an upregulation in binding site densities in the frontal cortex, olfactory tubercle, anterior nucleus, and striatum immediately after exposure, and these effects persisted at 30 days. Although red blood cell acetylcholinesterase (AChE) was not greatly inhibited by the 1-day exposure, there were 30 and 60% inhibitions after repeated exposures at the low and high doses, respectively. Histochemical staining for AChE demonstrated that sarin exposure alone reduced AChE in the cerebral cortex, striatum, and olfactory bulb. Sarin exposure under heat stress reduced AChE staining in the hippocampus, an area important for memory function. Thus, repeated exposures under heat-stress conditions, to levels of sarin that would not be noticed clinically, resulted in delayed development of brain alterations in cholinergic receptor subtypes that may be associated with memory loss and cognitive dysfunction.

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Husain et al. 1993. Delayed neurotoxic effect of sarin in mice after repeated inhalation exposure. *J Appl Toxicol.* 13(2):143-5.
Delayed neurotoxicity of sarin in mice after repeated inhalation exposure has been studied. Female mice exposed to atmospheric sarin (5 mg m-3 for 20 min) daily for 10 days developed muscular weakness of the limbs and slight ataxia on the 14th day after the start of the exposure. These changes were accompanied by significant inhibition of neurotoxic esterase (NTE) activity in the brain, spinal cord and platelets. Histopathology of the spinal cord of exposed animals showed focal axonal degeneration. These changes were comparatively less than in animals treated with the neurotoxic organophosphate, mipafox. Results from this study indicate that sarin may induce delayed neurotoxic effects in mice following repeated inhalation exposure.

Jakubowski et al. 2004. Quantitation of fluoride ion released sarin in red blood cell samples by gas chromatography-chemical ionization mass spectrometry using isotope dilution and large-volume injection. *J Anal Toxicol.* 28(5):357-63.
A new method for measuring fluoride ion released isopropyl methylphosphonofluoridate (sarin, GB) in the red blood cell fraction was developed that utilizes an autoinjector, a large-volume injector port (LVI), positive ion ammonia chemical ionization detection in the SIM mode, and a deuterated stable isotope internal standard. This method was applied

to red blood cell (RBC) and plasma ethyl acetate extracts from spiked human and animal whole blood samples and from whole blood of minipigs, guinea pigs, and rats exposed by whole-body sarin inhalation. Evidence of nerve agent exposure was detected in plasma and red blood cells at low levels of exposure. The linear method range of quantitation was 10-1000 pg on-column with a detection limit of approximately 2-pg on-column. In the course of method development, several conditions were optimized for the LVI, including type of injector insert, injection volume, initial temperature, pressure, and flow rate. RBC fractions had advantages over the plasma with respect to assessing nerve agent exposure using the fluoride ion method especially in samples with low serum butyrylcholinesterase activity.

Joosin et al. 2004. Cardiovascular effects of the adenosine A1 receptor agonist N6-cyclopentyladenosine (CPA) decisive for its therapeutic efficacy in sarin poisoning. *Arch Toxicol.* 78(1):34-9.
Mortality and occurrence of cholinergic symptoms upon sarin intoxication (144 micro g/kg s.c., approximately 2 x LD50) in rats is completely prevented by treatment with the adenosine A1 receptor agonist N6-cyclopentyladenosine (CPA, 2 mg/kg i.m.). Previously, we have shown that CPA treatment altered the distribution of sarin into the brain, presumably through its cardiovascular side effects. Therefore, the objective of the present study was to evaluate the contribution of the cardiodepressant effects of CPA to its therapeutic efficacy against sarin intoxication. Intramuscular treatment of rats with 0.5 and 2.0 mg/kg CPA 1 min after sarin poisoning attenuated most cholinergic symptoms and prevented mortality, which seemed to be directly associated with an immediate strong and long-lasting bradycardia and hypotension caused by CPA. Treatment with lower doses of CPA (0.1 and 0.05 mg/kg i.m.) caused similar levels of bradycardia and hypotension, albeit a few minutes later than at the higher doses of CPA. Upon sarin intoxication, this was correlated with increased incidence of cholinergic symptoms and decreased survival rates. Pretreatment with the peripheral adenosine A1 receptor antagonist 8- p-sulphophenyltheophylline (8-PST, 20 mg/kg i.p.) counteracted the cardiodepressant effects of 0.05 mg/kg CPA almost completely, thereby nearly abolishing its therapeutic efficacy against sarin poisoning. In conclusion, the present results strongly indicate that bradycardia and hypotension induced by the peripheral adenosine A1 receptor play a prominent role in the therapeutic efficacy of CPA in cases of sarin poisoning.

Kalra et al. 2002. Subclinical doses of the nerve gas sarin impair T cell responses through the autonomic nervous system. *Toxicol Appl Pharmacol.* 184(2):82-7.
The nerve gas sarin is a potent cholinergic agent, and exposure to high doses may cause neurotoxicity and death. Subclinical exposures to sarin have been postulated to contribute to the Gulf War syndrome; however, the biological effects of subclinical exposure are largely unknown. In this communication, evidence shows that subclinical doses (0.2 and 0.4 mg/m(3)) of sarin administered by inhalation to F344 rats for 1 h/day for 5 or 10 days inhibited the anti-sheep red blood cell antibody-forming cell response of spleen cells without affecting the distribution of lymphocyte subpopulations in the spleen. Moreover, sarin suppressed T cell responses, including the concanavalin A (Con A) and the anti-alphabeta-T cell receptor (TCR) antibody-induced T cell proliferation and the rise in the

intracellular calcium following TCR ligation. These concentrations of sarin altered regional but not total brain acetylcholinesterase activity. Interestingly, serum corticosterone levels of the sarin-treated animals were dramatically lower than the control animals, indicating that sarin-induced immunosuppression did not result from the activation of the hypothalamus-pituitary-adrenal (HPA) axis. Pretreatment of animals with the ganglionic blocker chlorisondamine abrogated the inhibitory effects of sarin on spleen cell proliferation in response to Con A and anti-TCR antibodies. These results suggest that the effects of sarin on T cell responsiveness are mediated via the autonomic nervous system and are independent of the HPA axis.

Kassa et al. 2000. Long-term alteration of immune functions following low level exposure to sarin in rats. *Acta Medica (Hradec Kralove).* 43(3):91-4.
1. Long term alteration of immune functions caused by low doses of nerve agent sarin were studied in rats exposed to sarin by inhalation. The alteration of immune functions by sarin was monitored by using two methods (the evaluation of in vitro spontaneous as well as stimulated proliferation of spleen cells and in vitro bactericidal activity of peritoneal macrophages) at 3, 6 and 12 months following sarin exposure. 2. The results indicate that not only symptomatic but also asymptomatic dose of sarin is able to alter some immune functions at six and twelve months following exposure to sarin. 3. Thus, not only organophosphorus insecticides but also nerve agents such as sarin can be potentially immunotoxic even at very low doses that do not cause clinically manifested intoxication following the inhalation exposure. The ability of sarin at low doses to alter immune functions seems to be really long term (up to 12 months following the exposure).

Kassa et al. 2004a. The alteration of immune reactions in inbred BALB/c mice following low-level sarin inhalation exposure. *Inhal Toxicol.* 16(8):509-15.
To study the influence of low-level sarin inhalation exposure on immune functions, inbred BALB/c mice were exposed to low concentrations of sarin for 60 min in the inhalation chamber. The evaluation of immune functions was carried out using phenotyping of CD3 (T lymphocytes), CD4 (helper T lymphocytes), CD8 (cytotoxic T lymphocytes), and CD19 cells (B lymphocytes) in the lungs, blood, and spleen, lymphoproliferation of spleen cells stimulated in vitro by various mitogens (concanavalin A, lipopolysaccharides), phagocyte activity of peritoneal and alveolar macrophages, production of N-oxides by peritoneal macrophages, and the measurement of the natural killer cell activity at 1 wk following sarin exposure. The results were compared to the values obtained from control mice exposed to pure air instead of sarin. The results indicate that low doses of sarin are able to alter the reaction of immune system at one week following exposure to sarin. While the numbers of CD3 cells in the lungs, blood, and spleen were slightly decreased, an increase in CD19 cells was observed, especially in the lungs and blood. The reduced proportion of T lymphocytes is caused by decay of CD4-positive T cells. Lymphoproliferation was significantly decreased regardless of the mitogen and sarin concentration used. The production of N-oxides by peritoneal macrophages was stimulated after exposure to the highest dose of sarin, whereas their ability to phagocytize the microbes was increased after exposure to the lowest dose of sarin. The natural killer cell activity was significantly higher in the case of inhalation

exposure of mice to the highest level of sarin. Thus, not only organophosphorus insecticides but also nerve agents such as sarin are able to alter immune functions even at a dose that does not cause clinically manifested disruption of cholinergic nervous system in the case of inhalation exposure. Nevertheless, the alteration of immune functions following the inhalation exposure to a symptomatic concentration of sarin seems to be more pronounced.

Kassa et al. 2004b. The influence of single or repeated low-level sarin exposure on immune functions of inbred BALB/c mice. *Basic Clin Pharmacol Toxicol.* 94(3):139-43.
To study the influence of single or repeated low-level sarin inhalation exposure on immune functions, inbred BALB/c mice were exposed to low clinically asymptomatic concentrations of sarin for 60 min. in the inhalation chamber. The evaluation of immune functions was carried out using phenotyping of CD3 (T-lymphocytes), CD4 (helper T-lymphocytes), CD8 (cytotoxic T-lymphocytes) and CD19 (B-lymphocytes) in the lungs, blood and spleen, lymphoproliferation of spleen cells stimulated in vitro by various mitogens (concanavalin A, lipopolysaccharides), phagocyte activity of peritoneal and alveolar macrophages, production of N-oxides by peritoneal macrophages and the measurement of the natural killer cell activity at one week after sarin exposure. The results were compared to the values obtained from control mice exposed to pure air instead of sarin. The results indicate that an asymptomatic dose of sarin is able to alter the reaction of the immune system at one week after exposure to sarin. While the number of CD3 cells in lung was significantly decreased, a slight increase in CD19 cells was observed especially in the lungs after a single sarin inhalation exposure. Lymphoproliferation was significantly decreased regardless of the mitogen and sarin concentration used and the number of low-level sarin exposures. The ability of peritoneal and alveolar macrophages to phagocyte the microbes was also decreased regardless of the number of low-level sarin exposures. The production of N-oxides by peritoneal macrophages was decreased following a single low-level sarin exposure but increased following repeated low-level sarin inhalation exposure. Nevertheless, the changes in the production of N-oxides that reflects a bactericidal activity of peritoneal macrophages was not significant. The natural killer cell activity was significantly higher in the case of inhalation exposure of mice to low concentration of sarin regardless of the number of exposures. Thus, not only organophosphorous insecticides but also nerve agents such as sarin are able to alter immune functions following a single inhalation exposure even at a dose that does not cause clinically manifested intoxication. Generally, the repeated exposure to low concentrations of sarin does not increase the alteration of immune functions compared to the single low-level sarin exposure with the exception of phagocyte activity of alveolar macrophages and natural killer cell activity.

Kassa 2002. Review of oximes in the antidotal treatment of poisoning by organophosphorus nerve agents. *J Toxicol Clin Toxicol.* 40(6):803-16.
The cholinesterase-inhibiting organophosphorus compounds referred to as nerve agents (soman, sarin, tabun, GF agent, and VX) are particularly toxic and are considered to be among the most dangerous chemical warfare agents. Included in antidotal medical countermeasures are oximes to reactivate the inhibited cholinesterase. Much experimental work has been done to better understand the properties of the oxime antidotal candidates

including the currently available pralidoxime and obidoxime, the H oximes HI-6 and Hlo-7, and methoxime. There is no single, broad-spectrum oxime suitablefor the antidotal treatment of poisoning with all organophosphorus agents. If more than one oxime is available, the choice depends primarily on the identity of the responsible organophosphorus compound. The H oximes appear to be very promising antidotes against nerve agents because they are able to protect experimental animals from toxic effects and improve survival of animals poisoned with supralethal doses. They appear more effective against nerve agent poisoning than the currently used oximes pralidoxime and obidoxime, especially in the case of soman poisoning. On the other hand, pralidoxime and especially obidoxime seem sufficiently effective to treat poisonings with organophosphorus insecticides that have relatively less toxicity than nerve agents.

Khan et al. 2000. Acute sarin exposure causes differential regulation of choline acetyltransferase, acetylcholinesterase, and acetylcholine receptors in the central nervous system of the rat. *Toxicol Sci.* 57(1):112-20.

Acute neurotoxic effects of sarin (O:-isopropylmethylphosphonoflouridate) in male Sprague-Dawley rats were studied. The animals were treated with intramuscular (im) injections of either 1 x LD(50) (100 microg/kg), and sacrificed at 0. 5, 1, 3, 6, 15, or 20 h after treatment, or with im injections of either 0.01, 0.1, 0.5, or 1 x LD(50) and sacrificed 15 h after treatment. Plasma butyrylcholinesterase (BChE) and brain regional acetylcholinesterase (AChE) were inhibited (45-55%) by 30 min after the LD(50) dose. BChE in the plasma and AChE in cortex, brainstem, midbrain, and cerebellum remained inhibited for up to 20 h following a single LD(50) treatment. No inhibition in plasma BChE activity was observed 20 h after treatment with doses lower than the LD(50) dose. Midbrain and brainstem seem to be most responsive to sarin treatment at lower doses, as these regions exhibited inhibition (approximately 49% and 10%, respectively) in AChE activity following 0.1 x LD(50) treatment, after 20 h. Choline acetyltransferase (ChAT) activity was increased in cortex, brainstem, and midbrain 6 h after LD(50) treatment, and the elevated enzyme activity persisted up to 20 h after treatment. Cortex ChAT activity was significantly increased following a 0.1 x LD(50) dose, whereas brainstem and midbrain did not show any effect at lower doses. Treatment with an LD(50) dose caused a biphasic response in cortical nicotinic acetylcholine receptor (nAChR) and muscarinic acetylcholine receptor (m2-mAChR) ligand binding, using [(3)H]cytisine and [(3)H]AFDX-384 as ligands for nAChR and mAChR, respectively. Decreases at 1 and 3 h and consistent increases at 6, 15, and 20 h in nAChR and m2-mAChR were observed following a single LD(50) dose. The increase in nAChR ligand binding densities was much more pronounced than in mAChR. These results suggest that a single exposure of sarin, ranging from 0.1 to 1 x LD(50), modulates the cholinergic pathways differently and thereby causes dysregulation in excitatory neurotransmission.

Laborde et al. 1996. Developmental toxicity of sarin in rats and rabbits. *JToxicol Environ Health.* 47(3):249-65.

Sarin (Agent GB, isopropyl methylphosphonofluoridate) is an organophosphate cholinesterase inhibitor. Sarin (Type I or Type II) was administered by gavage to CD rats on d 6-15 of gestation at dose levels of 0, 100, 240, or 380 micrograms/kg/d and to New

Zealand White (NZW) rabbits on d 6-19 of gestation at dose levels of 0, 5, 10, or 15 micrograms/kg/d. Females were weighed on gestational days (GD) 0, 6-16 for rats and 6-20 for rabbits, and immediately prior to termination (GD 20 for rats and GD 29 for rabbits). All animals were monitored daily for clinical signs of toxicity throughout dosing and until sacrifice. At necropsy, gravid uteri were weighed and examined for the number and status of implants (live, resorbed, or dead). Individual fetal body weight, malformations, and variations (external, visceral, and skeletal) were recorded. Rat and rabbit dams in the high-dose groups exhibited significant signs of maternal toxicity and increased maternal mortality. Examination of gravid uteri revealed no statistical differences among treatment groups in the incidence of resorptions or of dead or malformed fetuses, or in average body weight of live fetuses per litter. These results show no evidence or developmental toxicity in the CD rat or NZW rabbit following exposure to either Type I or Type II sarin during embryonic differentiation and major organogenesis, even at a dose that produced maternal toxicity.

Lee 2003. Clinical manifestations of sarin nerve gas exposure. *JAMA.* 290(5):659-62.

Li et al. 2000. The by-products generated during sarin synthesis in the Tokyo sarin disaster induced inhibition of natural killer and cytotoxic T lymphocyte activity. *Toxicology.* 146(2-3):209-20.
More than 5000 passengers on Tokyo subway trains were injured by the nerve gas, sarin and its by-products. Analysis of phosphor-carrying metabolites of sarin and its by-products in urine samples from the victims suggested that they were exposed not only to sarin, but also by-products generated during sarin synthesis, i.e. diisopropyl methylphosphonate (DIMP) and diethyl methylphosphonate (DEMP). We suspected genetic after-effects due to sarin by-products, thus, we checked the frequency of sister chromatid exchange (SCE) and found that SCE was significantly higher in the victims than in a control group, and that DIMP and DEMP significantly induced human lymphocyte SCE in vitro. In the present study, to explore whether DIMP and DEMP, which induced a high frequency of SCE of lymphocytes, also affected the lymphocyte functions, we examined the effect of DIMP and DEMP on splenic natural killer (NK) and splenic cytotoxic T lymphocyte (CTL) activity in mice, and NK activity of human lymphocytes in vitro. We found that DIMP and DEMP significantly inhibited NK and CTL activity in a dose-dependent manner. The inhibition induced by DIMP was stronger than that by DEMP. The effect of DIMP and DEMP on the splenic NK activity of mice was stronger than on the splenic CTL activity, and the human lymphocytes is more sensitive to DIMP and DEMP than the splenocytes of mice.

Li et al. 2002. Organophosphorus pesticides markedly inhibit the activities of natural killer, cytotoxic T lymphocyte and lymphokine-activated killer: a proposed inhibiting mechanism via granzyme inhibition. *Toxicology.* 172(3):181-90.
We have previously found that diisopropyl methylphosphonate, an organophosphorus by-product generated during sarin synthesis in the Tokyo sarin disaster, significantly inhibited natural killer (NK) and cytotoxic T lymphocyte (CTL) activities. In the present study, to investigate whether organophosphorus pesticides (OPs) also affect NK and CTL activities, we firstly examined the effect of five OPs on human NK activity, and then the

effect of Dimethyl 2,2-dichlorovinyl phosphate (DDVP), an OP on murine splenic NK, CTL and lymphokine-activated killer (LAK), and human LAK activities in vitro. To explore the underlying mechanism of decreased NK activity, we also investigated the effect of 4-(2-aminoethyl) benzenesulfonyl fluoride-HCl (p-ABSF), an inhibitor of serine proteases on NK, LAK and CTL activities, and the effect of DDVP on the activity of granzymes (serine proteases). We found that OPs significantly decreased human NK activity in a dose-dependent manner, but the degree of decrease in NK activity differed among the OPs investigated, and that DDVP significantly decreased NK, LAK and CTL activities in a dose-dependent manner, but the degree of decrease in these activities differed. p-ABSF showed a similar inhibitory pattern to DDVP, and had an additive inhibitory effect with DDVP on NK, LAK and CTL activities. We also found that DDVP significantly inhibited granzyme activity in a dose-dependent manner. These findings indicate that OPs significantly decrease NK, LAK and CTL activities in vitro via granzyme inhibition.

Li et al. 2004. Elevated frequency of sister chromatid exchanges of lymphocytes in sarin-exposed victims of the Tokyo sarin disaster 3 years after the event. *Toxicology.* 201(1-3):209-17.
We previously reported that the frequency of sister chromatid exchanges (SCEs) among victims of the Tokyo subway sarin disaster was significantly higher than that of controls 2-3 months after the disaster. It has been reported that the victims were also exposed to the by-products generated during sarin synthesis, i.e., diisopropyl methylphosphonate (DIMP), diethyl methylphosphonate (DEMP) and N,N-diethylaniline (DEA) during the disaster and we previously found that DIMP, DEMP and DEA induced a significant SCE increase in human lymphocytes in vitro. To monitor the genetic aftereffects of the sarin exposure, SCEs of peripheral blood lymphocytes were measured in fire fighters and police officers involved in the disaster 3 years after the event. We found that the frequency of SCEs was still significantly higher in the exposed subjects than the controls, suggesting a risk of the genetic aftereffects of the sarin exposure. We further found a significant positive correlation between the frequency of SCEs and the inhibition of serum cholinesterase activity in the exposed subjects, suggesting that the elevated frequency of SCEs is related to the sarin exposure. On the other hand, there was no significant difference in natural killer activity between the exposed and the controls.

Lindsay-Poland 1998. Toxic Aftertaste: The United States tested mustard gas on its own troops in Panama–and left a mess behind. *Progressive,* December 1998. Available at: http://www.progressive.org/lindsaypoland1298.htm

Maekawa et al. 1997. Genetic mutations of butyrylcholine esterase identified from phenotypic abnormalities in Japan. *Clin Chem.* 43(6 Pt 1):924-9.
We have identified 12 kinds of genetic mutations of butyrylcholine esterase (BCHE) from phenotypic abnormalities, showing that BCHE activities were deficient or diminished in sera. These genetic mutations, detected by PCR-single-strand conformation polymorphism analysis and direct sequencing, consisted of one deletion (BCHE*FS4), nine missense (BCHE*24 M, *1005, *250P, *267R, *330I, *365R, *418S, *515C,

*539T), and two nonsense mutations (BCHE*119STOP, *465STOP). All of the individuals deficient in serum BCHE activity were homozygous for silent genes (6 of 6). Fifty-eight percent of the individuals (31 of 53) with slightly reduced serum BCHE activity were heterozygous for silent genes. They also showed a higher frequency (47% as allele frequency) of the K-variant than the general population (17.5%). Finally, we confirmed low serum BCHE activity in 10 of 23 individuals heterozygous for silent genes.

Marrs 2003. Diazepam in the treatment of organophosphorus ester pesticide poisoning. *Toxicol Rev.* 22(2):75-81.
Although the main site of action of diazepam, as with other benzodiazepines, is at the gamma-aminobutyric acid A (GABAA) receptor, the degree to which the beneficial actions of diazepam in organophosphorus (OP) ester pesticide poisoning are mediated through the GABAA receptor has been a matter of controversy. Although in most series of OP intoxications, convulsions have been relatively uncommon, it is probable that convulsions produce long-term sequelae in the central nervous system by causing structural damage. Animal studies have demonstrated that diazepam prevents and treats convulsions produced by OPs and may prevent the late effects caused by damage to the central nervous system induced by such convulsions. Consequently, the use of diazepam is an important part of the treatment regimen of severe OP poisoning as it prevents, or at least reduces the duration of, convulsions. In addition, case reports suggest that diazepam will also ameliorate muscle fasciculation, a subjectively unpleasant feature of OP pesticide poisoning. There are no data, either experimental or clinical, demonstrating any clear effect of diazepam alone on lethality in OP poisoning. In fact, in one study of large animals, diazepam, given alone, increased lethality. In animals experimentally poisoned with OPs, combined treatment with atropine and diazepam significantly lowered lethality compared with atropine treatment alone, indicating a clear beneficial effect. There are numerous case reports of the use of diazepam, generally as an adjunct to other more specific OP antidotes such as atropine and/or pyridinium oximes. Based on this evidence and pharmacodynamic studies in experimental animals, diazepam should be given to patients poisoned with OPs whenever convulsions or pronounced muscle fasciculation are present. In severe poisoning, diazepam administration should be considered even before these complications develop. Although diazepam has a large therapeutic index, there appears to be no place for its routine use in OP poisoning. Diazepam should be given intravenously to patients treated in hospital for OP poisoning, although the intramuscular route is used to administer diazepam outside hospital, such as on the battlefield, when an auto-injector is employed. It should be recognised, however, that absorption by the intramuscular route is poor.

Matsuda et al. 1998. Detection of the sarin hydrolysis product in formalin-fixed brain tissues of victims of the Tokyo subway terrorist attack. *Toxicol Appl Pharmacol.* 150(2):310-20.
One of the hydrolysis products of sarin (isopropyl methylphosphonofluoridate) was detected in formalin-fixed brain tissues of victims poisoned in the Tokyo subway terrorist attack. Part of this procedure, used for the detection of sarin hydrolysis products in erythrocytes of sarin victims, has been described previously. The test materials were four

individual cerebellums, which had been stored in formalin fixative for about 2 years. Sarin-bound acetylcholinesterase (AChE) was solubilized from these cerebellums, purified by immunoaffinity chromatography, and digested with trypsin. Then the sarin hydrolysis products bound to AChE were released by alkaline phosphatase digestion, subjected to trimethylsilyl derivatization (TMS), and detected by gas chromatography-mass spectrometry. Peaks at m/z 225 and m/z 240, which are indicative of TMS-methylphosphonic acid, were observed within the retention time range of authentic methylphosphonic acid. However, no isopropyl methylphosphonic acid was detected in the formalin-fixed cerebellums of these 4 sarin victims, probably because the isopropoxy group of isopropyl methylphosphonic acid underwent chemical hydrolysis during storage. This procedure will be useful for the forensic diagnosis of poisoning by protein-bound, highly toxic agents, such as sarin, which are easily hydrolysed. This appears to be the first time that intoxication by a nerve agent has been demonstrated by analyzing formalin-fixed brains obtained at autopsy.

McGill et al. 2000. The "NRL-SAWRHINO": a nose for toxic gases. *Sensors and Actuators B.* 65:10-13.
At the Naval Research Laboratory (NRL), surface acoustic wave (SAW) chemical sensor systems have been in development since 1981. The primary focus has been the detection and identification of chemical agents and other toxic gases or vapors. In the recently developed "NRL-SAWRHINO" system (Rhino, Gr. Nose), a self-contained unit has been developed capable of autonomous field operation. An automated dual gas sampling system is included, for immediate and periodic detection capability. The latter, utilizes a trap-and-purge miniature gas chromatographic column, which serves to collect, concentrate, and separate vapor or gas mixtures prior to SAW analysis. The SAWRHINO includes all the necessary electronic and microprocessor control, SAW sensor temperature control, onboard neural net pattern recognition capability, and visual/audible alarm features for field deployment. The SAWRHINO has been trained to detect and identify a range of nerve and blister agents, and related simulants, and to discriminate against a wide range of interferent vapors and gases.

McDonough et al. 1997. Neuropharmacological mechanisms of nerve agent-induced seizure and neuropathology *Neurosci. Biobehav. Rev*. 21(5):559-79.
This paper proposes a three phase "model" of the neuropharmacological processes responsible for the seizures and neuropathology produced by nerve agent intoxication. Initiation and early expression of the seizures are cholinergic phenomenon; anticholinergics readily terminate seizures at this stage and no neuropathology is evident. However, if not checked, a transition phase occurs during which the neuronal excitation of the seizure per se perturbs other neurotransmitter systems: excitatory amino acid (EAA) levels increase reinforcing the seizure activity; control with anticholinergics becomes less effective; mild neuropathology is occasionally observed. With prolonged epileptiform activity the seizure enters a predominantly non-cholinergic phase: it becomes refractory to some anticholinergics; benzodiazepines and N-methyl--aspartate (NMDA) antagonists remain effective as anticonvulsants, but require anticholinergic co-administration; mild neuropathology is evident in multiple brain regions. Excessive influx of calcium due to repeated seizure-induced depolarization and prolonged

stimulation of NMDA receptors is proposed as the ultimate cause of neuropathology. The model and data indicate that rapid and aggressive management of seizures is essential to prevent neuropathology from nerve agent exposure.

Mientka 2002. DoD Acknowledges Civilian Exposure In SHAD Tests. *USMedicine* November 12, 2002. Available at: http://www.usmedicine.com/dailyNews.cfm?dailyID=114

Minami et al. 1997. Method for the analysis of the methylphosphonic acid metabolites of sarin and its ethanol-substituted analogue in urine as applied to the victims of the Tokyo sarin disaster. *J Chromatogr B Biomed Sci Appl*. 695(2):237-44.
An analysis method for the methylphosphonic acid metabolites of sarin in urine using trimethylsilyl derivatization and flame photometric detection is described in this report. Authentic reference standards of isopropyl methylphosphonic acid (IMPA) and ethyl methylphosphonic acid (EMPA) as well as methylphosphonic acid were employed to estimate the concentration in human urine. A sample pretreatment procedure was developed for urine using a column of cation-loaded ion-exchange resins (Ag+ -, Ba2+ - or H+ -Dowex) and adjusting the pH of the eluate from the column to 3.75-3.85 improved recovery of the target compounds. The eluate was evaporated to dryness under vacuum prior to trimethylsilylation, to remove water and any hydroxy- or amino-carrying volatile substances. The sarin metabolites, because of their low volatility, were concentrated and could be derivatized for analysis. The use of synthesized authentic sarin and ethylsarin metabolites, i.e., IMPA and EMPA, made it possible to establish the necessary sample pretreatment procedures for derivatization and gas chromatography-flame photometric detection (GC-FPD) analysis. The detection limits were 0.025 ppm both for EMPA and [MPA, and 0.625 microM for MPA, respectively. This method can be useful for estimating the exposure level to sarin by assaying the metabolites in urine and it is applicable to a large numbers of samples.

Minami et al. 1998. Biological monitoring of metabolites of sarin and its by-products in human urine samples. *J Toxicol Sci.* 23 Suppl 2:250-4.
More than 20,000 passengers of Tokyo underground trains were intoxicated with warfare toxic chemicals. Most of the patients examined had marked miosis and decreased serum cholinesterase activity. Transient increase of serum CPK activity after 3 days of the exposure was the another sign. We intensively analyzed the metabolites in the urine of 4 patients. The following analytic results indicated the exposure to sarin as well as contaminated compounds such as diisopropyl methylphosphonate (DIMP), ethyl methylphosphonate fluoridate (EMPF, or ethylsarin), diethyl methylphosphonate (DEMP), and ethyl isopropyl methylphosphonate (EIMP). (1) Isopropanol (IPA) and ethanol (EtOH) were detected of large quantities in the urine samples, and were thought to be derived from sarin and the sarin counterpart, EMPF, DIMP, DEMP and EIMP. (2) Monoalkyl methylphosphonic acids (isopropyl methylphosphonic acid (IMPA) and ethyl methylphosphonic acid (EMPA) also were excreted in large amounts with taking the similar excretion pattern of IPA and EtOH. (3) The metabolite only derived from sarin and ethylsarin is F anions whose integral output in the urine was less than the equimolar level of the excreted (IMPA + EMPA + IPA + EtOH). (4) Other corroborative findings

were low lethality: of more than 5,510 patients treated, 11 were acutely dead. (5) Nine exposed males had higher sister chromatid exchange (SCE) rate (5.00 +/- 1.48/cell) than the control (3.81 +/- 0.697/cell), because dialkyl methylphosphonates seemed to have alkylating activity and producing DNA adducts. The SCE rate also increased after the in vitro exposure of lymphocytes to dialkyl methylphosphonates.

Mitretek 2004. A Short History of the Development of Nerve Gases. Available at: http://www.mitretek.org/home.nsf/Homelandsecurity/HistoryNerveGases

Moore 1998. Long term health effects of low dose exposure to nerve agent. *J Physiology (Paris)* 92(3-4):325-8. Possible long-term toxic effects of nerve agents have been investigated using sensitive toxicological screens and extensive toxicity studies in various animal models. Data on humans have been obtained from controlled studies and accidental exposures. Studies in the area of 'low dose' exposure to nerve agents are currently being performed.

Morgan 1978. Jamaica ginger paralysis. Forty-seven year follow-up. *Arch. Neurol.* 35(8):530-532.
Eleven men who were victims of paralysis arising from ingestion of jake (Jamaica ginger extract) adulterated with lindol (isomers of cresyl phosphate) were examined. These men had taken the liquid in the early 1930's, when various batches of jake were distributed containing 0.5 to 3% tri-o-cresyl phosphate (TOCP). Nine of the eleven had consumed jake prior to this time without any ill effects. All the victims of these adulterated batches suffered some degree of paresis of both upper and lower extremities, usually with foot drop and wrist drop with clawed hands. All experienced complete or nearly complete recovery of hand and arm function, but only one of the 11 was able to later walk without use of assisting implements. Only one suffered any type of impotency for a time after the illness. TOCP poisoning destructively involves anterior horn cells and corticospinal tracts in the cord. Most of the evidence indicates that destruction of myelin sheaths is associated with axonal death. While these subjects showed some signs of frontal release and mild dementia, these could be attributed to advanced aged and cerebrovascular atherosclerosis. However, it is suggested that there is a possibility that TOCP may also affect cerebral function. TOCP closely resembles modern organophosphate pesticides, although it has little utility as a pesticide itself.

Morita et al. 1995. Sarin poisoning in Matsumoto, Japan. *Lancet.* 346(8970):290-3.
A presumed terrorist attack with sarin occurred in a residential area of the city of Matsumoto, Japan, on June 27, 1994. About 600 residents and rescue staff were poisoned; 58 were admitted to hospitals, and 7 died. We examined clinical and laboratory findings of 264 people who sought treatment and the results of health examinations on 155 residents done 3 weeks after the poisoning. Findings for severely poisoned people were decreases in serum cholinesterase, acetylcholinesterase in erythrocytes, serum triglyceride, serum potassium and chloride; and increases in serum creatine kinase, leucocytes, and ketones in urine. Slight fever and epileptiform abnormalities on electroencephalogram were present for up to 30 days. Examination revealed no persisting abnormal physical findings in any individual. Acetylcholinesterase returned to normal

within 3 months in all people examined. Although subclinical miosis and neuropathy were present 30 days after exposure, almost all symptoms of sarin exposure disappeared rapidly and left no sequelae in most people.

Moser et al. 2004. Placental Failure and Impaired Vasculogenesis Result in Embryonic Lethality for Neuropathy Target Esterase-Deficient Mice. *Mol Cell Biol.* 24(4): 1667–1679.
Age-dependent neurodegeneration resulting from widespread apoptosis of neurons and glia characterize the Drosophila Swiss Cheese (SWS) mutant. Neuropathy target esterase (NTE), the vertebrate homologue of SWS, reacts with organophosphates which initiate a syndrome of axonal degeneration. NTE is expressed in neurons and a variety of nonneuronal cell types in adults and fetal mice. To investigate the physiological functions of NTE, we inactivated its gene by targeted mutagenesis in embryonic stem cells. Heterozygous NTE+/− mice displayed a 50% reduction in NTE activity but underwent normal organ development. Complete inactivation of the NTE gene resulted in embryonic lethality, which became evident after gastrulation at embryonic day 9 postcoitum (E9). As early as E7.5, mutant embryos revealed growth retardation which did not reflect impaired cell proliferation but rather resulted from failed placental development; as a consequence, massive apoptosis within the developing embryo preceded its resorption. Histological analysis indicated that NTE is essential for the formation of the labyrinth layer and survival and differentiation of secondary giant cells. Additionally, impairment of vasculogenesis in the yolk sacs and embryos of null mutant conceptuses suggested that NTE is also required for normal blood vessel development.

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 releaase, 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.

Murata et al. 1997. Asymptomatic sequelae to acute sarin poisoning in the central and autonomic nervous system 6 months after the Tokyo subway attack. *J Neurol.* 244(10):601-6.
Six to eight months after the Tokyo subway attack in March 1995, the neurophysiological effects of acute sarin poisoning were investigated in 18 passengers exposed to sarin (sarin cases) in the subways to ascertain the focal or functional brain deficits induced by sarin. The event-related and visual evoked potentials (P300 and VEP), brainstem auditory evoked potential, and electrocardiographic R-R interval variability (CVRR), together with the score on the posttraumatic stress disorder (PTSD) checklist, were measured in the sarin cases and the same number of control subjects matched for sex and age. None of the sarin cases had any obvious clinical abnormalities at the time of testing. The P300 and VEP (P100) latencies in the sarin cases were significantly prolonged compared with the matched controls. In the sarin cases, the CVRR was significantly related to serum cholinesterase (ChE) levels determined immediately after exposure; the PTSD score was not significantly associated with any neurophysiological data despite the high PTSD score in the sarin cases. These findings suggest that asymptomatic sequelae to sarin exposure, rather than PTSD, persist in the higher and visual nervous systems beyond the turnover period of ChE; sarin may have neurotoxic actions in addition to the inhibitory action on brain ChE.

Nagao et al. 2003. Development of forensic diagnosis of acute sarin poisoning. Leg Med (Tokyo). 5 Suppl 1:S34-40.
On March 20, 1995, the Tokyo subway system was subjected to a horrifying terrorist attack with sarin gas (isopropyl methylphosphonofluoridate) that left 12 persons dead and over 5000 injured. In order to diagnose the definite cause of death of the victims, a new method was developed to detect sarin hydrolysis products in the erythrocytes and formalin-fixed cerebella from four victims of sarin poisoning. Sarin-bound acetylcholinesterase (AChE) was solubilized from the specimens of sarin victims and digested with trypsin. The sarin hydrolysis products bound to AChE were released by alkaline phosphatase digestion. The digested sarin hydrolysis products were subjected to trimethylsilyl derivatization and detected by gas chromatography-mass spectrometry. Sarin hydrolysis products were detected in all sarin poisoning victims.

Nakajima et al. 1999. Sequelae of sarin toxicity at one and three years after exposure in Matsumoto, Japan. *J Epidemiol.* 9(5):337-43.
In order to clarify the later sequelae of sarin poisoning that occurred in Matsumoto City, Japan, on June 27, 1994, a cohort study was conducted on all persons (2052 Japanese people) inhabiting an area 1050 meters from north to south and 850 meters from east to west with the sarin release site in the center. Respondents numbered 1237 and 836 people when surveys were conducted at one and three years after the sarin incident, respectively. Numbers of persons with symptoms of sarin toxicity were compared between sarin victims and non-victims. Of the respondents, 58 and 46 people had symptoms associated with sarin such as fatigue, asthenia, shoulder stiffness, asthenopia and blurred vision at both points of the survey, respectively. The prevalences were low; some complained of insomnia, had bad dreams, difficulty in smoking, husky voice, slight fever and palpitation. The victims who had symptoms one year after the incident had a lower

erythrocyte cholinesterase activity than did those who did not have symptoms at the early stage; such persons lived in an area with a 500 meter long axis north east from the sarin release site. The three-year cohort study clearly showed that the odds ratios of almost all of the symptoms were high in the sarin-exposed group, suggesting a positive relationship between symptoms and grades of exposure to sarin. These results suggest that symptoms reported by many victims of the sarin incident are thought to be sequelae related to sarin exposure.

Nakajima et al. 1998. Epidemiological study of sarin poisoning in Matsumoto City, Japan. *J Epidemiol.* 8(1):33-41.
On the night of June 27, 1994, about 12 liters of sarin were released by terrorists in Matsumoto City, Japan. In order to investigate the epidemic, community-based questionnaire surveys were conducted. The subjects were all inhabitants (2052 people) living and staying in an area of 1050 meters from north to south and 850 meters from east to west including the sarin release site. Participants included 1743 people who answered the questionnaire at the first survey; those with symptoms were contacted for follow-up at four months and one year after the episode. The number of sarin victims were 471 persons. Muscarinic signs were common to all victims; nicotinic signs were only seen in severely affected victims. The geographical distribution of sarin victims was closely related to the direction of the wind. Three weeks after the intoxication, 129 victims still had some symptoms such as dysesthesia of the extremities. At that time, many victims had begun to experience asthenopia, which was even more frequent at four months. Although victims who felt sarin-related symptoms had decreased by a year, some still had symptoms such as asthenopia. Sarin released in a suburban area affected approximately 500 inhabitants living nearby; some still had symptoms a year after the intoxication.

Nasr et al. 1988. SCE induction in Chinese hamster ovary cells (CHO) exposed to G agents. *Mutat Res.* 204(4):649-54.
Cultured Chinese hamster ovary (CHO) cells were exposed to two neurotoxic organophosphates, either sarin (GBI, GBII) at 1.4 X 10(-3) M or soman (GD) at 1.1 and 2.2 X 10(-3) M for 1 h, grown and their metaphase chromosomes scored for sister-chromatid exchanges (SCE). No cytotoxicity was seen with either agent at any dose level tested. Since histograms of SCE per cell showed that they were non-symmetrically arrayed around the mean, the number of SCEs were analyzed by using the nonparametric tests, Mann-Whitney and Kruskall-Wallis. Agents GBI and GBII did not show any significant increase in SCE over baseline. On the other hand, GD demonstrated a statistically significant increase in SCE with and without metabolic activation. Ethyl methanesulfonate (EMS) alone at 5 X 10(-3) M and cyclophosphamide (CP) at 10(-4) M in the presence of rat microsomes (S9) induced a 3- and 8-fold increase in SCE per cell, respectively.

National Research Council (US) 1997, *Committee on Toxicology, Subcommittee on Toxicity Values for Selected Nerve and Vesicant Agents, Review of Acute Human-Toxicity Estimates for Selected Chemical-Warfare Agents,* National Academy Press, Washington, D.C. Available at: http://www.nap.edu/readingroom/books/toxicity/

National Research Council (US) 1999. Health Risk Assessment for The Nerve Agent GB. *In 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 B, National Academy Press. Available at http://www.nap.edu/books/0309065984/html/131.html

Newmark 2004a. The birth of nerve agent warfare: lessons from Syed Abbas Foroutan. *Neurology*. 62(9):1590-6.
The author reviewed Farsi-language articles published recently by Dr. Syed Abbas Foroutan, which constitute the only firsthand clinical descriptions of battlefield nerve agent casualties in the world literature, and the author compares his comments with US and North Atlantic Treaty Organization (NATO) chemical casualty care doctrine. Foroutan's lessons learned reassure us that a robust medical evacuation system, coupled with timely and appropriate medical care of nerve agent poisoning, will save many more lives on future battlefields.

Niven et al. 2004. Inhalational exposure to nerve agents. *Respir Care Clin N Am.* 10(1):59-74.
The respiratory system plays a major role in the pathogenesis of nerve agent toxicity. It is the major route of entry and absorption of nerve agent vapor, and respiratory failure is the most common cause of death follow-ing exposure. Respiratory symptoms are mediated by chemical irritation,muscarinic and nicotinic receptor overstimulation, and central nervous system effects. Recent attacks have demonstrated that most patients with an isolated vapor exposure developed respiratory symptoms almost immediately. Most patients had only mild and transient respiratory effects, and those that did develop significant respiratory compromise did so rapidly. These observations have significant ramifications on triage of patients in a mass-casualty situation, because patients with mild-to-moderate exposure to nerve agent vapor alone do not require decontamination and are less likely to develop progressive symptoms following initial antidote therapy. Limited data do not demonstrate significant long-term respiratory effects following nerve agent exposure and treatment. Provisions for effective respiratory protection against nerve agents is a vital consideration in any emergency preparedness or health care response plan against a chemical attack.

Noort et al. 1998. Quantitative analysis of O-isopropyl methylphosphonic acid in serum samples of Japanese citizens allegedly exposed to sarin: estimation of internal dosage. *Arch Toxicol.* 72(10):671-5.
A convenient and rapid micro-anion exchange liquid chromatography (LC) tandem electrospray mass spectrometry (MS) procedure was developed for quantitative analysis in serum of O-isopropyl methylphosphonic acid (IMPA), the hydrolysis product of the nerve agent sarin. The mass spectrometric procedure involves negative or positive ion electrospray ionization and multiple reaction monitoring (MRM) detection. The method could be successfully applied to the analysis of serum samples from victims of the Tokyo subway attack and of an earlier incident at Matsumoto, Japan. IMPA levels ranging from 2 to 135 ng/ml were found. High levels of IMPA appear to correlate with low levels of residual butyrylcholinesterase activity in the samples and vice versa. Based on our

analyses, the internal and exposure doses of the victims were estimated. In several cases, the doses appeared to be substantially higher than the assumed lethal doses in man.

Nozaki et al. 1997. Relationship between pupil size and acetylcholinesterase activity in patients exposed to sarin vapor. *Intensive Care Med.* 23(9):1005-7.
OBJECTIVE: To elucidate the effect of sarin vapor on pupil size and erythrocyte acetylcholinesterase activity (AchE). DESIGN: Retrospective observational survey. SETTING: Emergency department of an urban teaching hospital. PATIENTS: 80 patients who were exposed to sarin in a terrorist attack in Tokyo subways. MEASUREMENTS AND RESULTS: Pupil size and AchE activity on the day of exposure were measured. Among the 80 patients, the pupils were miotic (< 3 mm) in 50 patients (62.5%), while AchE activity was below the normal range (< 1.2 U) in 34 patients (42.5%). AchE was significantly lower in the miotic group than in the group with normal pupils (1.0 +/- 0.5 U vs 1.5 +/- 0.3 U, $p < 0.01$). In the miotic group, AchE activity was lower than normal in 32 patients (64.0%) but was decreased in only 2 patients in the normal pupil group (6.7%) ($p < 0.01$). CONCLUSIONS: Miosis is a more sensitive index of exposure to sarin vapor than erythrocyte AchE. Systemic poisoning is apparently less likely to develop if the patient's pupil size is normal on arrival at the hospital.

Ohbu et al. 1997. Sarin poisoning on Tokyo subway. *South Med J.* 90(6):587-93.
On the day of the disaster, 641 victims were seen at St. Luke's International Hospital. Among those, five victims arrived with cardiopulmonary or respiratory arrest with marked miosis and extremely low serum cholinesterase values; two died and three recovered completely. In addition to these five critical patients, 106 patients, including four pregnant women, were hospitalized with symptoms of mild to moderate exposure. Other victims had only mild symptoms and were released after 6 hours of observation. Major signs and symptoms in victims were miosis, headache, dyspnea, nausea, ocular pain, blurred vision, vomiting, coughing, muscle weakness, and agitation. Almost all patients showed miosis and related symptoms such as headache, blurred vision, or visual darkness. Although these physical signs and symptoms disappeared within a few weeks, psychologic problems associated with posttraumatic stress disorder persisted longer. Also, secondary contamination of the house staff occurred, with some sort of physical abnormality in more than 20%.

Okudera 2002. Clinical features on nerve gas terrorism in Matsumoto. *J Clin Neurosci.* 9(1):17-21.
Clinical features on the first unexpected nerve gas terrorism using sarin (isopropyl methylphosphonofluoridate) on citizens in the city of Matsumoto is described. The nerve gas terrorism occurred at midnight on 27 June, 1994. About 600 people including residents and rescue staff were exposed to sarin gas. Fifty-eight victims were admitted to hospitals and seven died. Theoretically, sarin inhibits systemic acetylcholinesterase and damages all the autonomic transmission at the muscarinic and nicotinic acetylcholine receptor. Miosis was the most common finding in the affected people. In cases with severe poisoning, organophosphate may affect the central nervous system and cause cardiomyopathy. A few of the victims complained of arrhythmia and showed a decreased cardiac contraction. Abnormal electroencephalograms were recorded in two patients. The

clinical features and follow-up studies are discussed with reference to the Tokyo subway terrorism and related articles.

Okudera et al. 1997. Unexpected nerve gas exposure in the city of Matsumoto: report of rescue activity in the first sarin gas terrorism. *Am J Emerg Med.* 15(5):527-8
This report describes the rescue activities and the exposure of rescue and hospital personnel from the first unexpected nerve gas terrorist attack using sarin (isopropyl methylphophonofluoridate) in the city of Matsumoto at midnight on June 27, 1994. The details of the emergency activities in the disaster were studied based on the records from emergency departments of the affiliated hospitals and records from the firehouse. About 600 people, including residents and rescue staff, were exposed to sarin gas. Fifty-eight residents were admitted to hospitals, and 7 died. Among 95 rescuers and the duty doctor from the doctor car, 8 had mild symptoms of poisoning. All the rescue activity took place without gas masks or decontamination procedures. In this case of unexpected mass exposure to sarin gas, the emergency rescue system for a large disaster in Matsumoto city, which had been established for a conflagration or a local earthquake, was effective.

Okumura et al. 2000. Lessons Learned from the Tokyo Subway Sarin Attack. *Prehosp Disast Med* 15(3):s30.
On the morning of 20 March 1995, sarin was released in the Tokyo Subway System. There had never been such a large scale act of urban terrorism using a nerve gas. There are many lessons to be learned from Tokyo Subway Sarin Attack. Two major lessons can be cited in summary:
1) Absence of decontamination - In total, 1,364 EMTs were dispatched, and among them, 135 were secondarily affected. At St. Luke's hospital, 23% of the medical staff complained of symptoms and signs of secondary exposure. Fortunately, nobody died from the secondary exposure. The religious cult used a 30% sarin solution. If they had used a 100% sarin solution, the outcome would have been much more tragic - secondarily exposed prehospital and medical staff would have been killed. This is the reason for the development of decontamination facilities and the use of personal protective equipment (PPE) in the prehospital and hospital settings; and
2) Confusion of information and lack of coordination among related organizations - Japan is a highly vertically structured society. Fire departments, police, metropolitan governments, and hospitals acted independently without coordination. After the attack, the Japanese government developed the Severe Chemical Hazard Response Team.
The Prime Minister's office created a National Security and Crisis Management Office that calls realistic desktop hazmat drills involving the concerned organizations and specialists

Okumura et al. 1998a. The Tokyo subway sarin attack: disaster management, Part 1: Community emergency response. *Acad Emerg Med.* 5(6):613-7.
The Tokyo subway sarin attack was the second documented incident of nerve gas poisoning in Japan. Prior to the Tokyo subway sarin attack, there had never been such a large-scale disaster caused by nerve gas in peacetime history. This article provides details related to how the community emergency medical services (EMS) system responded from the viewpoint of disaster management, the problems encountered, and how they

were addressed. The authors' assessment was that if EMTs, under Japanese law, had been allowed to maintain an airway with an endotracheal tube or use a laryngeal mask airway without physician oversight, more patients might have been saved during this chemical exposure disaster. Given current legal restrictions, advanced airway control at the scene will require that doctors become more actively involved in out-of-hospital treatment. Other recommendations are: 1) that integration and cooperation of concerned organizations be established through disaster drills; 2) that poison information centers act as regional mediators of all toxicologic information; 3) that a real-time, multidirectional communication system be established; 4) that multiple channels of communication be available for disaster care; 5) that public organizations have access to mobile decontamination facilities; and 6) that respiratory protection and chemical-resistant suits with gloves and boots be available for out-of-hospital providers during chemical disasters.

Okumura et al. 1998b. The Tokyo subway sarin attack: disaster management, Part 2: Hospital response. *Acad Emerg Med.* 5(6):618-24.
The Tokyo subway sarin attack was the second documented incident of nerve gas poisoning in Japan. The authors report how St. Luke's Hospital dealt with this disaster from the viewpoint of disaster management. Recommendations derived from the experience include the following: Each hospital in Japan should prepare an emergent decontamination area and have available chemical-resistant suits and masks. Ventilation in the ED and main treatment areas should be well planned at the time a hospital is designed. Hospital disaster planning must include guidance in mass casualties, an emergency staff call-up system, and an efficient emergency medical chart system. Hospitals should establish an information network during routine practice so that it can be called upon at the time of a disaster. The long-term effects of sarin should be monitored, with such investigation ideally organized and integrated by the Japanese government.

Organisation for the Prohibition of Chemical Weapons (OPCW) 2004. Chemical Terrorism in Japan: The Matsumoto and Tokyo Incidents. Available at: http://www.opcw.org/resp/html/japan.html

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.

Parascandola 1995. The Public Health Service and Jamaica ginger paralysis in the 1930s. *Public Health Rep*. 110(3):361-3.

Peterson 2002. Lessons from Iran on facing chemical war: Scientists and doctors visit Iran to gain expertise on handling chemical attacks. *Christian Science Monitor* November 19, 2002. Available at: http://www.csmonitor.com/2002/1119/p01s03-wome.html.

PIER 2004. Nerve Agent Exposure > Diagnosis. *American College of Physicians, Physicians Information and Educational Resource.* Available at: http://pier.acponline.org/physicians/public/d890/diagnosis/d890-g3.5.html

Polhuijs et al. 1997. New method for retrospective detection of exposure to organophosphorus anticholinesterases: application to alleged sarin victims of Japanese terrorists. *Toxicol Appl Pharmacol.* 146(1):156-61.
With regard to detection of exposure to anticholinesterase, the presently used methods have the disadvantage that they cannot detect either low-level exposures with certainty or the structure of the agent and the extent of poisoning. In principle, organophosphate-inhibited butyrylcholinesterase in human plasma is the most persistent and abundant source for biomonitoring of exposure to organophosphate anticholinesterases. Fluoride ions reactivate the inhibited enzyme readily at pH 4, converting the organophosphate moiety into the corresponding phosphofluoridate. Subsequent quantitation of the latter product provides a reliable, highly sensitive and retrospective method for detection of exposure to, or handling of, organophosphates such as nerve agents and organophosphorus pesticides. We applied the new procedure to serum samples from victims of the Tokyo subway attack by the AUM Shinriyko sect and from an earlier incident at Matsumoto. In serum of 10 of 11 victims from the Tokyo incident and of 2 of the 7 samples from the Matsumoto incident, reactivation with fluoride ions yielded sarin concentrations in the range of 0.2-4.1 ng/ml serum. Evidently, these victims had been exposed to an organophosphate with the structure PriO(CH3)P(O)X, presumably with X = F (sarin). Several applications of the new procedure to establish nerve agent and/or organophosphate (OP) pesticide exposure can be envisaged, e.g., (i) in biomonitoring of exposure for health surveillance of those handling organophosphates, (ii) in cases of alleged exposure to nerve agents and/or OP pesticides in armed conflict situations or terrorist attacks, (iii) in medical treatment of intoxication, and (iv) in forensic cases against suspected terrorists that may have handled anticholinesterases.

Quistad et al. 2003. Evidence that mouse brain neuropathy target esterase is a lysophospholipase. *Proc Natl Acad Sci USA.* 100(13):7983–7987.
Neuropathy target esterase (NTE) is inhibited by several organophosphorus (OP) pesticides, chemical warfare agents, lubricants, and plasticizers, leading to OP-induced delayed neuropathy in people (>30,000 cases of human paralysis) and hens (the best animal model for this demyelinating disease). The active site region of NTE as a

recombinant protein preferentially hydrolyzes lysolecithin, suggesting that this enzyme may be a type of lysophospholipase (LysoPLA) with lysolecithin as its physiological substrate. This hypothesis is tested here in mouse brain by replacing the phenyl valerate substrate of the standard NTE assay with lysolecithin for an "NTE-LysoPLA" assay with four important findings. First, NTE-LysoPLA activity, as the NTE activity, is 41–45% lower in Nte-haploinsufficient transgenic mice than in their wild-type littermates. Second, the potency of six delayed neurotoxicants or toxicants as in vitro inhibitors varies from IC50 0.02 to 13,000 nM and is essentially the same for NTE-LysoPLA and NTE (r2 = 0.98). Third, the same six delayed toxicants administered i.p. to mice at multiple doses inhibit brain NTE-LysoPLA and NTE to the same extent (r2 = 0.90). Finally, their in vivo inhibition of brain NTE-LysoPLA generally correlates with delayed toxicity. Therefore, OP-induced delayed toxicity in mice, and possibly the hyperactivity associated with NTE deficiency, may be due to NTE-LysoPLA inhibition, leading to localized accumulation of lysolecithin, a known demyelinating agent and receptor-mediated signal transducer. This mouse model has some features in common with OP-induced delayed neuropathy in hens and people but differs in the neuropathological signs and apparently the requirement for NTE aging.

Raveh et al. 1993. Human butyrylcholinesterase as a general prophylactic antidote for nerve agent toxicity. In vitro and in vivo quantitative characterization. *Biochem Pharmacol.* 45(12):2465-74.
Butyrylcholinesterase purified from human plasma (HuBChE) was evaluated both in vitro and in vivo in mice and rats as a single prophylactic antidote against the lethal effects of highly toxic organophosphates (OP). The variation among the bimolecular rate constants for the inhibition of HuBChE by tabun, VX, sarin, and soman was 10-fold (0.47 to 5.12 x 10(7) M-1 min-1; pH 8.0, 26 degrees). The half-life of HuBChE in blood after its i.v. administration in mice and rats was 21 and 46 hr, respectively. The peak blood-enzyme level was obtained in both species approximately 9-13 hr following i.m. injection of HuBChE, and the fraction of the enzyme activity absorbed into the blood was 0.9 and 0.54 for rats and mice, respectively. The stoichiometry of the in vivo sequestration of the anti-cholinesterase toxicants was consistent with the HuBChE/OP ratio of the molar concentration required to inhibit 100% enzyme activity in vitro. Linear correlation was demonstrated between the blood level of HuBChE and the extent of protection conferred against the toxicity of nerve agents. Pretreatment with HuBChE alone was sufficient not only to increase survivability following exposure to multiple median lethal doses of a wide range of potent OPs, but also to alleviate manifestation of toxic symptoms in mice and rats without the need for additional post-exposure therapy. It appeared that in order to confer protection against lethality nerve agents had to be scavenged to a level below their median lethal dose LD50 within less than one blood circulation time. Since the high rate of sequestration of nerve agents by HuBChE is expected to underlie the activity of the scavenger in other species as well, a reliable extrapolation of its efficacy from experimental animals to humans can be made.

Rengstorff 1985. Accidental exposure to sarin: vision effects. *Arch Toxicol.* 56(3):201-3.
Two men were accidentally exposed to vapors of sarin, a cholinesterase inhibitor and extremely toxic nerve gas. Diagnosis was confirmed by depressed cholinesterase activity,

and fixed extremely miotic pupils. No other signs or symptoms developed and neither man required treatment. Recovery to normal cholinesterase activity was gradual over a 90-day period. Pupillary reflexes were not detectable until 11 days after exposure; the miotic pupils dilated slowly over a 30-45 day-period. Eye pain and blurred vision did not occur; visual acuity and amplitude of accommodation were improved for several weeks. Other functions not affected significantly were intraocular pressure, visual fields, color vision, heterophorias, and vergences.

Ritter 2004. Iraq Sarin Shell is not Part of a Secret Cache. *Christian Science Monitor* May 21, 2004. Available at: http://www.commondreams.org/views04/0521-06.htm

Rostker 1997. Information Paper: M8A1 Automatic Chemical Agent Alarm. Department of Defense. Available at: http://www.gulflink.osd.mil/m8a1alarms/

Scremin et al. 2003. Delayed neurologic and behavioral effects of subtoxic doses of cholinesterase inhibitors. *J Pharmacol Exp Ther.* 304(3):1111-9.
We tested the hypothesis that pyridostigmine bromide (PB) intake and/or low-level sarin exposure, suggested by some as causes of the symptoms experienced by Persian Gulf War veterans, induce neurobehavioral dysfunction that outlasts their effects on cholinesterase. Adult male Sprague-Dawley rats were treated during 3 weeks with s.c. saline, PB in drinking water (80 mg/l), sarin (62.5 microg/kg; 0.5x LD(50), three times/week s.c.), or PB in drinking water + sarin. Animals were tested for passive avoidance, nociceptive threshold, acoustic startle, and open field activity 2, 4, or 16 weeks after treatment. Two weeks after sarin, acoustic startle was enhanced, whereas distance explored in the open field decreased. These effects were absent with PB + sarin or PB by itself. No effect on any variable was found at 4 weeks, whereas at 16 weeks sarin induced a decrease and PB + sarin induced an increase in habituation in the open field test. Nociceptive threshold was elevated in the PB + sarin group at 16 weeks. No effect of treatment on passive avoidance was noted in any group. Brain regional acetylcholinesterase and cholineacetyltransferase activities were not affected at any time after treatment, but muscarinic receptors were down-regulated in hippocampus, caudate putamen, and mesencephalon in the sarin group at 2 weeks. In conclusion, this study gives further support to the use of PB against nerve agent poisoning and does not support the hypothesis that delayed symptoms experienced by Persian Gulf War veterans could be due to PB, alone or in association with low-level sarin exposure.

Senanayake et al. 1987. Neurotoxic effects of organophosphorus insecticides. An intermediate syndrome. *N Engl J Med.* 316(13):761-3.
Acute neurotoxic effects during the cholinergic phase of organophosphorus insecticide poisoning and delayed neurotoxic effects appearing two to three weeks later are well recognized. We observed 10 patients who had paralysis of proximal limb muscles, neck flexors, motor cranial nerves, and respiratory muscles 24 to 96 hours after poisoning, after a well-defined cholinergic phase. The compounds involved were fenthion, monocrotophos, dimethoate, and methamidophos. Four patients urgently required ventilatory support. The paralytic symptoms lasted up to 18 days. A delayed polyneuropathy later developed in one patient. Three patients died. Electromyographic

studies showed fade on tetanic stimulation, absence of fade on low-frequency stimulation, and absence of post-tetanic facilitation, suggestive of a postsynaptic defect. This neuromuscular junctional defect may have been the predominant cause of the paralytic symptoms, with neural and central components contributing to various degrees. Our patients appeared to have a distinct clinical entity (a so-called intermediate syndrome) that developed after the acute cholinergic crisis and before the expected onset of the delayed neuropathy.

Seto 2001. The sarin gas attack in Japan and the related forensic investigation. *Organisation for the Prohibition of Chemical Weapons* Summer/June 2001. Available at: http://www.opcw.org/synthesis/html/s6/p14prt.html.

Sidell et al. 1992. Chemical warfare agents: II. Nerve agents. *Ann Emerg Med.* 21(7):865-71.
Nerve agents are highly potent and rapidly acting organophosphorus compounds that irreversibly bind and inactive acetylcholinesterase. Only rarely have they been used in warfare, but their great lethality and the threat that they pose have encouraged production and stockpiling in large quantities. They differ in a number of important ways from common agricultural organophosphate insecticides. In light of recent threats of chemical warfare and the possibilities of chemical acts of terrorism, North American physicians should be knowledgeable of the effects of these agents and the care of exposure victims.

Sidell 1997. Nerve Agents. In *Medical Aspects of Chemical and Biological Warfare*. (Sidell FR, Takafuji ET, Franz DR Eds.) Chapter 5, Office of the Surgeon General, Dept. of the Army, United States of America.
Available at http://www.bordeninstitute.army.mil/cwbw/Ch5.pdf

Sidell et al. 1997. Longterm Health Effects of Nerve Agents and Mustard. In *Medical Aspects of Chemical and Biological Warfare*. (Sidell FR, Takafuji ET, Franz DR Eds.) Chapter 8, Office of the Surgeon General, Dept. of the Army, United States of America.
Available at http://www.bordeninstitute.army.mil/cwbw/Ch8.pdf

Silveira et al. 1990. Putative M2 muscarinic receptors of rat heart have high affinity for organophosphorus anticholinesterases. *Toxicol Appl Pharmacol.* 103(3):474-81.
The M2 subtype of muscarinic receptor is predominant in heart, and such receptors were reported to be located in muscles as well as in presynaptic cholinergic and adrenergic nerve terminals. Muscarinic receptors of rat heart were identified by the high affinity binding of the agonist (+)-[3H]cis-methyldioxolane ([3H]CD), which has been used to label a high affinity population of M2 receptors. A single population of sites (KD 2.74 nM; Bmax of 82 fmol/mg protein) was detected and [3H]CD binding was sensitive to the M2 antagonist himbacine but much less so to pirenzepine, the M1 antagonist. These cardiac receptors had different sensitivities to NiCl2 and N-ethylmaleimide from brain muscarinic receptors, that were also labeled with [3H]CD and considered to be of the M2 subtype. Up to 70% of the [3H]CD-labeled cardiac receptors had high affinities for several organophosphate (OP) anticholinesterases. [3H]CD binding was inhibited by the nerve agents soman, VX, sarin, and tabun, with K0.5 values of 0.8, 2, 20, and 50 nM,

respectively. It was also inhibited by echothiophate and paraoxon with K0.5 values of 100 and 300 nM, respectively. The apparent competitive nature of inhibition of [3H]CD binding by both sarin and paraoxon suggests that the OPs bind to the acetylcholine binding site of the muscarinic receptor. Other OP insecticides had lower potencies, inhibiting less than 50% of 5 nM [3H]CD binding by 1 microM of EPN, coumaphos, dioxathion, dichlorvos, or chlorpyriphos. There was poor correlation between the potencies of the OPs in reversibly inhibiting [3H]CD binding, and their anticholinesterase activities and toxicities. Acetylcholinesterases are the primary targets for these OP compounds because of the irreversible nature of their inhibition, which results in building of acetylcholine concentrations that activate muscarinic and nicotinic receptors and desensitize them, thereby inhibiting respiration. Nevertheless, the high affinities that cardiac muscarinic receptors have for these toxicants point to their extra vulnerability. It is suggested that the success of iv administration of the muscarinic receptor inhibitor atropine in initial therapy of poisoning by OP anticholinesterases may be related in part to the extra sensitivity of M2 receptors to certain OPs.

Singer et al. 1987. Cardiomyopathy in Soman and Sarin intoxicated rats. *Toxicol Lett.* 36(3):243-9.
Rats surviving various single dose of the organophosphorus anticholinesterase nerve agents Soman and Sarin were examined by light microscopy at intervals up to 35 days post-exposure. Brain lesions, identical to those that have been reported elsewhere were present, as well as a previously unreported finding associated with Soman or Sarin intoxication: half of all animals that had brain lesions also had areas of myocardial degeneration and necrosis. Depending upon the point in time at which cardiac tissues were examined, findings varied from areas of acute myolysis and necrosis to areas undergoing resolution of damage. The finding of brain lesions in those animals having cardiac lesions suggests a relationship between the convulsion induced neurologic and cardiac lesions. These studies suggest that convulsive doses of chemical warfare agents induce pathological changes in the cardiovascular system of laboratory animals.

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.

Smith et al. 1930. The pharmacological action of certain phenol esters with special reference to the etiology of so-called ginger paralysis. *Public Health Rep.* 45:2509–24.

Spencer et al. 2000. Sarin, Other "Nerve Agents," and Their Antidotes. In *Experimental and Clinical Neurotoxicology 2nd Ed.* (Spencer PS, Schaumburg HH, Eds.) Oxford University Press, New York pp 1073-1093.

Spruit et al. 2000. Intravenous and inhalation toxicokinetics of sarin stereoisomers in atropinized guinea pigs. *Toxicol Appl Pharmacol*. 169(3):249-54.
We report the first toxicokinetic studies of (+/-)-sarin. The toxicokinetics of the stereoisomers of this nerve agent were studied in anesthetized, atropinized, and restrained

guinea pigs after intravenous bolus administration of a dose corresponding to 0.8 LD50 and after nose-only exposure to vapor concentrations yielding 0.4 and 0.8 LCt50 in an 8-min exposure time. During exposure the respiratory minute volume and frequency were monitored. Blood samples were taken for gas chromatographic analysis of the nerve agent stereoisomers and for measurement of the activity of blood acetylcholinesterase (AChE). In all experiments, the concentration of (+)-sarin was below the detection limit (<5 pg/ml). The concentration-time profile of the toxic isomer, i.e., (-)-sarin, after an intravenous bolus was adequately described with a two-exponential equation. (-)-Sarin is distributed ca. 10-fold faster than C(-)P(-)-soman, whereas its elimination proceeds almost 10-fold slower. During nose-only exposure to 0.4 and 0.8 LCt50 of (+/-)-sarin in 8 min, (-)-sarin appeared to be rapidly absorbed. The blood AChE activity decreased during the exposure period to ca. 15 and 70% of control activity, respectively. There were no effects on the respiratory parameters. A significant nonlinearity of the toxicokinetics with dose was observed for the respiratory experiments.

Street et al. 1975. Alteration of induced cellular and humoral immune responses by pesticides and chemicals of environmental concern: quantitative studies of immunosuppression by DDT, aroclor 1254, carbaryl, carbofuran, and methylparathion. *Toxicol Appl Pharmacol.* 32(3):587-602.
Dose-dependent, immunosuppressive effects of continued dietary treatment of rabbits with DDT, Aroclor 1254, carbaryl, carbofuran, and methylparathion were studied. The animals were given a diet containing graded amounts of chemicals for 4 wk and challenged with sheep red blood cells and Freund's adjuvant. The testing followed for an additional 4 wk while the animals were maintained on the same diets as before. The most sensitive indication of immunosuppression was based on evaluation of lymphatic organs, primarily those dependent on thymus-derived lymphocytes. The chemical treatments resulted in a decreased count of plasma cells in popliteal lymph nodes (except with carbaryl), reduction of germinal centers in the spleen, and increasing atrophy of thymus cortex. These responses were generally scaled to increasing levels of the compounds tested. Hemolysin and hemagglutinin titers were not significantly affected by any of the chemical treatments nor were consistent trends observed. The antigen-induced increase in serum gamma-globulin was consistently decreased with DDT, Aroclor, carbaryl, and carbofuran treatments, but only carbaryl produced significant changes (at 10 days postantigen). DDT groups showed significantly higher preantigen gamma-globulin values which were less evident following antigen challenge. Skin sensitivity to tuberculin was decreased (except with carbaryl) but generally only at high dosages of the test chemicals. None of the compounds showed any effect on growth, food consumption, leucocyte count, or on organ to body weight ratios for liver, kidney, spleen, and adrenal, except for slight liver enlargement caused by Aroclor 1254.

Suziki et al. 1997. Eighteen cases exposed to sarin in Matsumoto, Japan. *Intern Med.* 36(7):466-70.
Forty-six patients who were exposed to sarin consulted our hospital because of darkness of vision, and ocular pain, vomiting, dyspnea and headaches on June 27 and 28, 1994. Eighteen patients were admitted and 4 of them were in the critical state. There were 6 features: 1) depression of plasma cholinesterase activity (17 of 18 patients, 94%), 2)

hypokalemia (4/18, 22%), 3) depression of triglyceride (12/18, 67%), 4) hypocapnia (5/17, 29%), 5) partial pressure of oxygen (PaO2) <80 mmHg, or requirement of O2 inhalation (15/18, 83%), 6) white blood cells (WBC) >9,000 per mm3 (13/18, 72%). Seventeen patients were discharged from hospital, but one patient is still suffering from akinetic mutism after two years.

Tochigi et al. 2002. Serum cholesterol, uric acid and cholinesterase in victims of the Tokyo subway sarin poisoning: a relation with post-traumatic stress disorder. *Neurosci Res.* 44(3):267-72.
Cholesterol and uric acid, which might correlate with steroidogenesis and monoamine functions, may change under emotionally stressful conditions and in mental disturbances. Among anxiety disorders, an increase of serum cholesterol has been observed in panic disorder. However, the issue has not been adequately investigated in other anxiety disorders, including post-traumatic stress disorder (PTSD). The present study investigated serum cholesterols, uric acid and cholinesterase in victims of the Tokyo subway sarin poisoning, 1995, in a series of 5-year follow-ups. Cholinesterase was studied, in relevance with serum lipid changes and symptoms of PTSD, and also in light of a biological effect of sarin. Out of 34 victims, eight developed PTSD and two were currently diagnosed with PTSD using the Clinician-Administered PTSD Scale (CAPS). No significant relationship was observed between PTSD and serum cholesterols or uric acid. Several factors including co-occurrence of other mental disturbances with PTSD, in addition to the limited sample size, might have affected the result. In contrast, serum cholinesterase level was significantly reduced in the victims with the development of PTSD, compared with the matched controls ($P<0.02$, t-test). This might partly reflect a long-term remnant effect of sarin intoxication, although an effect of the psychological experience could not be totally excluded.

Trovero et al. 1998. Pharmacological profile of CEB-1957 and atropine toward brain muscarinic receptors and comparative study of their efficacy against sarin poisoning. *Toxicol Appl Pharmacol.* 150(2):321-7.
This study consists of two parts, first to compare the pharmacological profile of atropine and CEB-1957 substance toward muscarinic receptor subtypes. In various rat brain structures, binding properties were determined by competition experiments of [3H]pirenzepine, [3H]AF-DX 384, and [3H]4-DAMP in quantitative autoradiography of M1, M2, and M3 muscarinic receptor subtypes, respectively. Competition curves have shown that atropine presents similar nanomolar inhibition constants toward each subtype, while CEB-1957 has distinct affinities (Ki from 0.26 to 73 nM) with the following range order: M3 > or = M2 > M1. The second part is to compare atropine and CEB-1957 (in combination with pralidoxime) for their ability to protect against the lethality induced by 2 x LD50 of the acetylcholinesterase inhibitor sarin. CEB-1957 reduced the mortality at doses 10 times lower than atropine. Finally, from these results, it is proposed that a selective blockade of M2 and M3 receptor subtypes could play a pivotal role in the protective effect against sarin poisoning.

Tuovinen 2004. Organophosphate-induced convulsions and prevention of neuropathological damages. *Toxicology.* 196(1-2):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

Tuovinen et al. 1999. Success of pyridostigmine, physostigmine, eptastigmine and phosphotriesterase treatments in acute sarin intoxication. *Toxicology.* 134(2-3):169-78. The acute toxicity of organophosphorus (OP) compounds in mammals is due to their irreversible inhibition of acetylcholinesterase (AChE) in the nervous system, which leads to increased synaptic acetylcholine levels. The protective actions of intravenously (i.v.) administered pyridostigmine, physostigmine, eptastigmine, and an organophosphate hydrolase, phosphotriesterase, in acute sarin intoxication were studied in mice. The acute intragastric (i.g.) toxicity (LD50) of sarin with and without the pretreatments was tested by the up-and-down method. The mice received pyridostigmine (0.06 mg/kg body weight), physostigmine (0.09 mg/kg body weight), the physostigmine derivative eptastigmine (0.90 mg/kg body weight) or phosphotriesterase (104 U/g, 10.7 microg/g body weight) 10 min prior to the i.g. administration of sarin. Physostigmine was also administered with phosphotriesterase. Phosphotriesterase was the most effective antidote in sarin intoxication. The LD50 value for sarin increased 3.4-fold in mice receiving phosphotriesterase. Physostigmine was the most effective carbamate in sarin exposure. The protective ratios of physostigmine and pyridostigmine were 1.5- and 1.2-1.3-fold, respectively. Eptastigmine did not give any protection against sarin toxicity. Both the phosphotriesterase and physostigmine treatments protected the brain AChE activities measured 24 h after sarin exposure. In phosphotriesterase and physostigmine-treated mice, a 4- and 2-fold higher sarin dose, respectively, was needed to cause a 50% inhibition of brain AChE activity. Moreover, the combination of phosphotriesterase-physostigmine increased the LD50 value for sarin 4.3-fold. The animals pretreated with phosphotriesterase-ephysostigmine tolerated four times the lethal dose in control animals, furthermore their survival time was 2-3 h in comparison to 20 min in controls. In conclusion, phosphotriesterase and physostigmine were the most effective treatments

against sarin intoxication. However, eptastigmine did not provide any protection against sarin toxicity.

WHO 2004. Chemical Terrorism in Japan: The Matsumoto and Tokyo Incidents. In *Health Aspects of Biological and Chemical Weapons, 2nd edition* Available at http://www.opcw.org/resp/html/japan.html

Wiener et al. 2004. Nerve Agents: A Comprehensive Review *J Intensive Care Med.* 19(1):22-37
Nerve agents are perhaps the most feared of potential agents of chemical attack. The authors review the history, physical characteristics, pharmacology, clinical effects, and treatment of these agents. Available at: http://jic.sagepub.com/cgi/framedreprint/19/1/22

Wilson et al. 2002. Actions of pyridostigmine and organophosphate agents on chick cells, mice, and chickens. *Drug Chem Toxicol.* 25(2):131-9.
Gulf War veterans were given pyridostigmine bromide (PB) tablets to enhance the therapeutic effect of antidotes to nerve agents in the event of exposure. The goal of this research is to examine whether combined exposure to PB and sarin (agent GB) is more neurotoxic to sensitive surrogate animals, mice and chickens, than if given separately. Scoping trials were performed to establish appropriate dose-response ranges for sarin and control chemicals. IC50 values were determined in chickens and mice for in vitro inhibition of acetylcholinesterase (AChE) and neuropathy target esterase (NTE). The results indicated PB neither inhibits NTE nor does it spare sarin's inhibition of AChE. Chick embryo nerve cells in vitro showed more inhibition of AChE activity and no faster recovery when PB treatment was followed by DFP treatment than the other way around. Experiments on chickens also indicated that PB treatment did not inhibit NTE and that it crossed the blood brain barrier inhibiting brain AChE although to a lesser extent than it inhibited blood cholinesterases. Other experiments determined multiple dose levels in chickens for sarin and DFP that inhibited > 80% of NTE, considered a threshold for triggering organophosphate-induced delayed neuropathy.

Winkenwerder 2002a. Technical Report: Modeling and Risk Characterization of US Demolition Operations at the Khamisiyah Pit. APPENDIX B – Hazard Identification (Detailed Description)-Tab BI Chemical properties. Department of Defense Available at: http://www.gulflink.osd.mil/khamisiyah_tech/kham_tech_tabbi.htm - appbTABBIChemicalPropertiesofSarinandCyc

Winkenwerder 2002b. Case Narrative: US Demolition Operations at Khamisiyah Final Report. Available at http://www.gulflink.osd.mil/khamisiyah_iii/index.htm

Veterans Administration 2004. Project 112 and Project SHAD (Deseret Test Center) Pocket Guide. Available at: http://www1.va.gov/shad/docs/RevisedProject112-SHADPocketCardJan04.pdf

Yamasaki 1997. The Arg192 isoform of paraoxonase with low sarin-hydrolyzing activity is dominant in the Japanese. ***Hum Genet.*** **101(1):67-8.**
The high-density-lipoprotein-associated enzyme paraoxonase, which has a role in the detoxification of organophosphorus compounds, is known to be polymorphic in humans. The Arg192 isoform of paraoxonase hydrolyzes paraoxon more rapidly than the Gln192 isoform. However, with respect to the hydrolysis of toxic nerve agents, such as diazoxon, soman, and sarin, the Arg192 isoform displays a lower activity than the other isoform. To evaluate the possibility that the genetic polymorphism was involved in the aggravated extent of human injury in the sarin gas poisoning incident in the Tokyo subway in March 1995, we investigated the prevalance of this polymorphism in the Japanese population. We found that the Arg192 allele is more common in the Japanese (allele frequency: 0.66) than in people of other races (ranging 0.24-0.31). In the Japanese, 135 out of the 326 subjects (41.4%) investigated were homozygous for the Arg192 allele, which shows a very low hydrolysis activity for sarin. Thus, there seems to be a racial difference in vulnerability to toxic nerve agents, such as sarin. The dominance of the Arg192 allele in the Japanese population probably worsened the tragedy of March 1995 in the Tokyo subway.

Yamasue et al. 2003. Voxel-based analysis of MRI reveals anterior cingulate gray-matter volume reduction in posttraumatic stress disorder due to terrorism. *Proc Natl Acad Sci U S A.* 100(15):9039-43.
MRI studies using the manual tracing method have shown a smaller-than-normal hippocampal volume in patients with posttraumatic stress disorder (PTSD). However, these studies have yielded inconsistent results, and brain structures other than the hippocampus have not been well investigated. A recently developed, fully automated method called voxel-based morphometry enables an exploration of structural changes throughout the brain by applying statistical parametric mapping to high-resolution MRI. Here we first used this technology in patients with PTSD. Participants were 9 victims of the Tokyo subway sarin attack with PTSD and 16 matched victims of the same traumatic event without PTSD. The voxel-based morphometry showed a significant gray-matter volume reduction in the left anterior cingulate cortex (ACC) in trauma survivors with PTSD compared with those without PTSD. The severity of the disorder was negatively correlated with the gray-matter volume of the left ACC in PTSD subjects. There were no significant differences in other gray-matter regions or any of the white-matter regions between two groups. The present study demonstrates evidence for structural abnormalities of ACC in patients with PTSD. Together with previous functional neuroimaging studies showing a dysfunction of this region, the present findings provide further support for the important role of ACC, which is pivotally involved in attention, emotional regulation, and conditioned fear, in the pathology of PTSD.

Yanno et al. 1997. Neuroparalytic agent acute poisoning and its long-term effects. Meditsina Truda I Promyshlennaya Ekologiya; 6:5-7.
The authors analyzed over 200 cases of acute poisoning with sarin**,** soman and VX chemical, determined risk of the poisoning in various conditions. The clinical manifestations of acute poisoning and the long-term effects are presented.

Yokoyama et al. 1998. Chronic neurobehavioral effects of Tokyo subway sarin poisoning in relation to posttraumatic stress disorder. *Arch Environ Health.* 53(4):249-56.
Chronic neurobehavioral effects of acute sarin poisoning were evaluated in 9 male and 9 female patients who were exposed to sarin poisoning in the Tokyo subway incident in Japan. The investigators used nine neurobehavioral tests, as well as a posttraumatic stress disorder checklist, 6-8 mo after the poisoning occurred. Serum cholinesterase activity in patients on the day of poisoning (i.e., March 20, 1995) ranged from 13 to 131 IU/l (mean=72.1 IU/l). The results of analysis covariance, in which age, education level, alcohol consumption, and smoking status (covariates) were controlled in 18 sarin cases and in 18 controls, showed that the score on the digit symbol (psychomotor performance) test was significantly lower in the sarin cases than in controls. Nonetheless, the scores for the General Health Questionnaires, fatigue of Profile of Mood States, and posttraumatic stress disorder checklist were significantly higher in the sarin cases than controls. The investigators added posttraumatic stress disorder to the covariates, and only the score on the digit symbol test was significantly lower in sarin cases. In addition, the results of stepwise multiple regression analysis in 18 sarin cases revealed that scores for the General Health Questionnaires, fatigue of Profile of Mood States (i.e., fatigue, tension-anxiety, depression, and anger-hostility)-together with the paired-associate learning test-were associated significantly with posttraumatic stress disorder. The association did not remain significant for the digit symbol test score. Perhaps a chronic effect on psychomotor performance was caused directly by acute sarin poisoning; on the other hand, the effects on psychiatric symptoms (General Health Questionnaire) and fatigue (Profile of Mood States) appeared to result from posttraumatic stress disorder induced by exposure to sarin.

Zabrodskii et al. 2003. Anticholinesterase mechanism as a factor of immunotoxicity of various chemical compounds. *Bull Exp Biol Med.* 136(2):176-8.
Experiments on Wistar rats showed that acute poisoning with chemicals in a dose of 0.75 LD(50) (dimethyl dichlorovinyl phosphate, sarin, VX substance, sulfur yperite, lewisite, tetraethyl lead, dichloroethane) inhibiting platelet acetylcholine esterase, alpha-naphthyl-AS-acetate esterase, and alpha-naphthyl-butyrate esterase suppressed T cell-mediated immune reactions.

Zacharias 2002. Depot studies. Utah incident. *Tri-City Herald* 8/18/2002.