Therapeutics & Toxins News

Newsletter for the TDM and Toxicology Division of AACC

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Chemical Warfare Agents: a Century of Terror; Part 2 – the Nerve Agents

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Previously, in the January 2015 issue, we began a discussion about chemical weapons that focused on the vesicants, a group of blister agents that were extensively used during World War I about a century ago (1). Now, we will complete this series by discussing the more modern and lethal chemical weapons, the nerve agents. Specifically, we will focus on the four major nerve agents: tabun, sarin, soman and VX.

The nerve agents are the most potent of all the chemical warfare agents. Sadly, over the last thirty years they have been used in both military and nonmilitary settings with significant consequences. The first nerve agents were discovered by German chemists prior to the start of World War II while searching for more potent organophosphate (OP) insecticides. Tabun was synthesized in 1936 by Gerhard Schrader but was soon abandoned due to its remarkable toxicity to humans. Sarin was subsequently synthesized in 1938 followed by soman in 1944. The Nazi government recognized their military potential and directed the chemical company IG Farben to secretly mass produce tabun and sarin. Thankfully, these agents were never used; when Allied forces discovered them at the end of the war they were astonished. The Allies then conducted their own research and created code names for the new German (G) series of nerve agents based upon when they were developed. The G agent designations were GA for tabun, GB for sarin and GD for soman. After the war a vast array of far less toxic OPs were commercially developed as insecticides and ever since they have had a critical role in agriculture. To date, more than 200 OPs have been formulated into thousands of different insecticides used worldwide; some common types include parathion, malathion, chlorpyrifos, diazinon, and dursban (2).

In 1952, a British chemical company inadvertently synthesized an even more potent OP while searching for a dichlorodiphenyltrichloroethane (DDT) replacement. This compound was sent to the U.S. where it was developed into the nerve agent VX. During the Cold War nearly all major industrial powers developed their own nerve agent stockpiles with the U.S. and Soviet Union having the most extensive. The Soviets spent considerable resources to be able to operate in a chemical laden battlefield; their arsenal was estimated to be ten times larger than the Americans. Accidents occurred, the most notable happened in an area adjacent to Dugway Proving Ground, Utah. In March 1968 a VX leak drifted out of an Army test area during aerial spraying and killed over 6,000 sheep grazing several miles downwind in Skull Valley (3). The first recorded intentional use occurred in the 1980s Iran-Iraq War when Saddam Hussein's forces used large quantities of tabun and sarin against the Iranians resulting in 45,000 - 120,000 casualties (3). In the closing days of this war, Iraqi forces also attacked the Kurdish village of Halabja with nerve agents and vesicants killing several thousand civilians. In 1994 - 1995, the doomsday cult Aum Shinrikyo conducted two sarin attacks on Japanese civilians that resulted in over 1,200 casualties and 20 deaths. The most notorious attack occurred in the Tokyo subway system during rush hour that was followed by a long delay in identifying sarin as the poison because clinicians wrongly assumed the gas involved was cyanide or carbon monoxide. More recently, the Syrian government in 2013 used sarin-filled munitions against insurgents near Damascus which prompted international intervention.
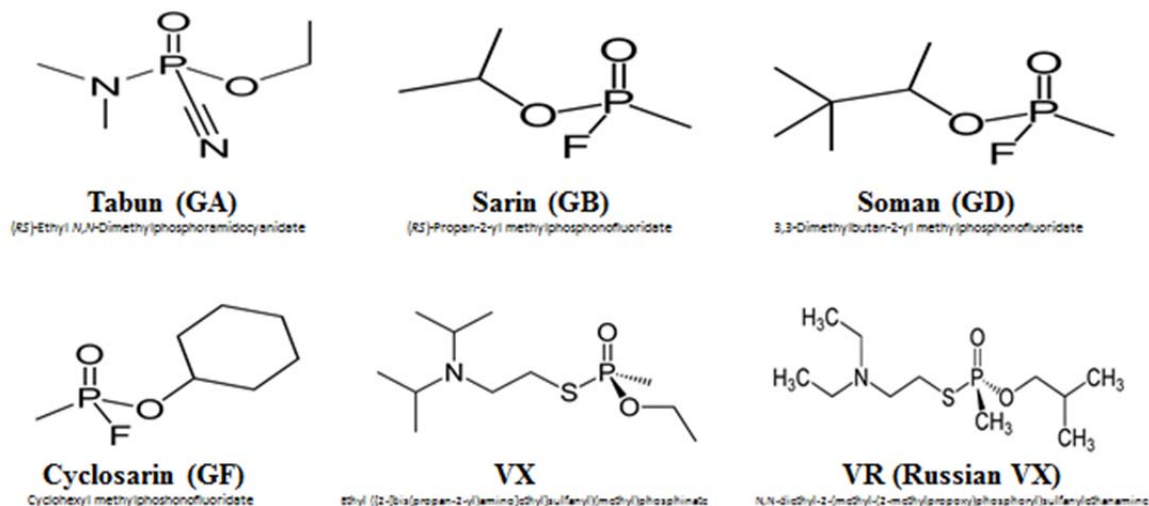
Nerve Agent Properties: All nerve agents are classified as organophosphates (OPs), a general term for esters of phosphoric acid, phosphorus acid and phosphinic acid. Figure 1 illustrates their chemical structures along with common and IUPAC names while Table 1 lists some of their key properties. LCt50 is defined as the estimated dosage that would be lethal to 50% of unprotected humans per cubic meter over one minute. MCt50 is similar but reflects the dosage that causes miosis (i.e. pinpoint pupils). At normal temperatures and pressures nerve agents are not gases but liquids that can be efficiently dispersed as aerosols. They are some of the most toxic substances known to man; a dermal exposure of just 10 mg of VX can be lethal to an adult human. The nerve agents differ in their potential toxicities but they are all far more toxic than the OP insecticides. For instance, sarin is 1,000-fold more toxic than parathion. The G agents are relatively volatile and present a significant vapor hazard, especially sarin. VX is much more viscous with a consistency like motor oil. It is more readily absorbed through the skin than the G agents and has a lengthy environmental persistence such that it could tactically be used to limit terrain access. Pure nerve agents are clear and colorless; but impure solutions may exhibit different properties. Reports indicate tabun may appear as a brownish liquid with a fruity smell, soman can also smell fruity or like oil of camphor and VX may be straw-colored (3).

Many other nerve agents have been developed and tested beyond these three G agents and VX. Two noteworthy compounds are cyclosarin and Russian VX. Cyclosarin is an analog of sarin first developed by the Germans and designated as GF (3). After World War II it was discounted by the Allies as being of limited utility until interest was renewed during the Persian Gulf War. Due to difficulties in receiving chemicals needed to produce sarin, the Iraqi government opted to make cyclosarin. They found that it evaporates much more slowly than sarin so they often mixed them together to create a more persistent agent. It has a toxicity intermediate between sarin and tabun. Russian VX was developed by the Soviets in the late 1950s and given the designation VR (3). It was mass produced and preliminary testing suggests it has a comparable level of toxicity as VX.

Table 1. Key Properties of the Nerve Agents

Nerve Agent	Tabun	Sarin	Soman	VX
LD50 (liquid) in a 70 kg man	1,000 mg	1,700 mg	350 mg	10 mg
LCt50 (vapor) mg-min/m^3	400	100	50	10
MCt50 (vapor) mg-min/m^3	2-3	3	<1	0.04
Volatility (at 25^0 C) mg/m^3	610	22,000	3,900	10.5
Aging T1/2 h = hours; min = minutes	13.3-14 h	3-5 h	2-6 min	48 h

Figure 1. Chemical Structures of the Nerve Agents.

Tabun (GA)
(RS)-Ethyl N,N-Dimethylphosphoramidocyanidate

Sarin (GB)
(RS)-Propan-2-yl methylphosphonofluoridate

Soman (GD)
3,3-Dimethylbutan-2-yl methylphosphonofluoridate

Cyclosarin (GF)
Cyclohexyl methylphosphonofluoridate

VX
Ethyl ({2-[bis(propan-2-yl)amino]ethyl}sulfanyl)(methyl)phosphinate

VR (Russian VX)
N,N-diethyl-2-(methyl-(2-methylpropoxy)phosphoryl)sulfanylethanamine

OP Insecticides: While these compounds are far less toxic than nerve agents they still represent a major global health problem. Annually, OP insecticides are involved in an estimated 3 million poisonings; approximately one-third from accidental exposures and the remainder often involve suicide attempts. This translates into about 200,000 fatalities each year (2). Recently a school headmistress in Bihar, India was found to have poisoned the student lunch with an OP pesticide resulting in 23 deaths (4). Due to safety concerns their use in the U.S. has declined significantly since the 1980s, often being replaced with less toxic carbamates. However, they still account for about one-third of all pesticides used. While the relative toxicity across the spectrum of OPs differs greatly, they all have a similar mode of action.

Toxic Mechanism: The enzyme acetylcholinesterase (AChE, EC 3.1.1.7) has a critical role in terminating nerve transmission by rapidly hydrolyzing acetylcholine (ACh) present in the synapse. AChE is expressed primarily at neuromuscular junctions in the peripheral nervous system (PNS) and cholinergic synapses in the central nervous system (CNS.) It is also found in the lungs, spleen, the gray matter of the brain, and on the surface of red blood cells. The nerve agents and OP insecticides directly inhibit AChE by binding to the active site at a critical serine. This prevents the ACh substrate from binding which then accumulates in the synapse and can precipitate a cholinergic toxidrome. Once in the active site the nerve agents can then lose an alkyl group and form a stable covalent bond with the serine. This process is

called aging and causes irreversible enzyme inhibition. The length of time this takes to occur varies, it can be as short as a few minutes for soman or take several days for VX.

Cholinergic Toxidrome: The major consequence of AChE inhibition is a cholinergic crisis caused by the increased levels of ACh. The excess neurotransmitter stimulates cholinergic muscarinic receptors at neuronal synapses in the PNS and CNS and stimulates then depresses cholinergic nicotinic receptors at neuromuscular junctions. Diagnosis is based solely on the clinical presentation of the victims and not on laboratory assays. The mnemonic DUMB BELSS is often used to remember the commonly observed effects on the muscarinic system. It stands for Diarrhea, Urination, Miosis, Bradycardia, Bronchorrhea/ Bronchoconstriction, Emesis, Lacrimation, Sweating, and Salivation. The resultant toxidrome can also include nicotinic symptoms that start with tachycardia and muscle fasciculations followed by weakness that can lead to flaccid paralysis. The signs and symptoms observed will depend on the balance between the overloaded muscarinic and nicotinic receptors; some consider the combination of miosis and muscle fasciculations to be pathognomonic of OP exposure (5). Aerosol or vapor exposure primarily affects the eyes, nose and respiratory tract and commonly manifests as miosis and bronchoconstriction. In contrast, dermal exposures often produce localized muscle fasciculations and diaphoresis without miosis. Regardless of the exposure route, the more severely intoxicated will likely present with emesis, apnea, seizures and/or be unconscious. The principal cause of death is hypoxia due to bronchorrhea and bronchoconstriction. G agents typically have an onset of symptoms from seconds to minutes while VX can develop hours post-exposure.

Lab Analysis and Detection: There are two different, but related, cholinesterase enzymes present in the blood; an AChE form that is GPI-anchored to the surface of red blood cells (RBC-AChE) and serum butyrylcholinesterase (BChE, EC 3.1.1.8). Both can be used as surrogate markers for neuronal AChE inhibition confirming the initial clinical diagnosis of a nerve agent poisoning. Serum BChE, sometimes referred to as pseudocholinesterase, is often used to monitor acute poisonings because its activity declines and returns to normal more rapidly than RBC-AChE. The latter is more commonly used to detect chronic exposures in workers who have potential contact with OPs such as agricultural field hands or laboratory personnel. These individuals should have an initial baseline level determined and then be periodically tested to monitor for OP exposure. The most commonly used commercial assays measure their enzyme activity via the Ellman chromogenic method which is fast, accurate and inexpensive. Briefly, this method involves the functional cholinesterase in the sample hydrolyzing a thiocholine ester substrate into an intermediate containing a free sulfhydryl. This then reacts with dithiobis-2-nitrobenzoic acid to form a yellow colored product that is spectrophotometrically measured at 400 - 420 nm. These assays have several limitations that

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are described thoroughly elsewhere along with some other less commonly employed cholinesterase methods (2). After severe nerve agent exposures both BChE and RBC-AChE activity levels can become undetectable.

Nerve agents can be routinely detected in biological (urine or blood) and environmental samples via GC-MS and LC-MS/MS (6). The G agents are readily hydrolyzed resulting in half-lives measured in minutes while the more persistent VX can be detected for several hours. In humans, these compounds are believed to be extensively metabolized by serum esterases that form products readily excreted in the urine (7). Alkyl-phosphonic acids are often used as specific biomarkers and some metabolites can be present for several days to weeks post-exposure (3). Several validated MS quantitative methods have demonstrated limits of detection at pg/mL levels and dilute-and-shoot LC-MS/MS methods have been successfully developed for urinalysis (8).

Treatments: Three different classes of pharmaceutical agents are essential in the management of nerve agent exposures: anticholinergics, oximes and benzodiazepines. The standard anticholinergic antidote is atropine which acts as an effective antagonist at cholinergic muscarinic receptors. It can alleviate the classic cholinergic toxidrome described above and is administered parenterally, either by an intravenous (IV) or intramuscular (IM) route. Repeat doses are given until a therapeutic endpoint is achieved, typically the clearing of bronchial secretions and/or resolution of bronchoconstriction. Of note, pulse and pupil diameters are not useful for monitoring the response to atropine.

Often atropine treatment is accompanied by an oxime. These highly nucleophilic substances can dephosphorylate the inhibited AChE if given prior to enzyme aging. They are administered by IV or IM routes and can reverse the neuromuscular nicotinic effects as the regenerated AChE normalizes neurotransmission. In the U.S., the only oxime widely available is pralidoxime (2-PAM) but other oximes confer varying degrees of protection. The U.S. military developed and employs a nerve agent antidote autoinjector kit that contains 2 mg of atropine and 600 mg of 2-PAM (Figure 2). These autoinjectors permit efficient IM injection through clothing and the rapid absorption of both antidotes. Similar antidote kits are sold commercially.

Benzodiazepines are currently the best treatment for seizures induced by severe nerve agent exposures and are administered by IV or IM. The U.S. military employs a diazepam (10 mg) autoinjector (Figure 2). Current policy is to administer it prior to seizures becoming evident if three or more nerve agent antidote kits were previously used. Other conventional antiseizure medications such as phenytoin, are considered ineffective.

Figure 2. From top to bottom, pralidoxime & atropine autoinjectors, diazepam autoinjector. Chemical structures are presented on the right.
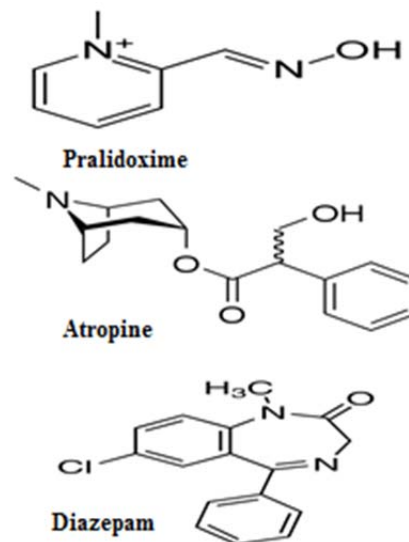
The timely administration of atropine and oximes may be insufficient to protect against soman since it can very rapidly age AChE. In just 6 minutes after a severe exposure the majority of a person's AChE can be permanently inhibited. To address this significant issue a carbamate pretreatment was developed. Carbamates are AChE inhibitors that transiently occupy the active site thereby blocking access to nerve agents and conferring protection. This binding is spontaneously reversible; they do not age AChE and are easily displaced by oximes, regenerating functional enzyme. American troops in the Persian Gulf War orally ingested 30 mg of the carbamate pyridostigmine bromide every 8 hours when under threat of nerve agent attack (3). The U.S. military has also investigated recombinant human BChE as another pretreatment (9). While BChE has no known physiological role it is generally thought to serve as a molecular scavenger capable of binding anticholinergics like the nerve agents thereby minimizing their effects on the life sustaining AChE (2, 3).

In response to an incident involving nerve agents, one should immediately minimize exposure by distancing victims from the source. Decontamination then starts with the complete removal of all contaminated clothing and jewelry. This should be done outside of healthcare facilities as secondary exposures commonly occurred among hospital personnel in both Japanese attacks (10). Further, it is recommended that the removed clothing be placed somewhere airtight like sealed plastic bags due to the potential off gassing of the G agents. The exposed should then be washed with copious amounts of water or a 1:10 dilute bleach solution in water along with soap and a gentle brush. Rapid washing is more important than the cleaning solution used as it takes from 15 - 20 minutes for bleach solutions to inactivate chemicals. Another lesson learned in the Tokyo sarin attack is to anticipate mass hysteria

after an event; about 80% of patients seen at hospitals did not exhibit signs or symptoms of nerve agent exposure (3).

The world has come a long way over the last century since the German Army released the first crude chemical weapons in World War I. Instead of relatively insidious non-lethal chemicals used on the battlefield we are now much more concerned with highly lethal nerve agents being employed by transnational terrorists. Recently, ISIS used sulfur mustard and chlorine gas in Syria and it seems not if but when nerve agents will be employed by a group like this. Thankfully, most countries have banned together to contain this menace by ratifying the Chemical Weapons Convention (11). This international pact mandates that all signatories fully disclose their chemical weapon stockpiles and puts their arsenal under the control of the Organization for the Prohibition of Chemical Weapons. They ensure that the Convention has been honored through inspections and document the destruction of chemical weapons. Their success in these efforts resulted in them being awarded the 2013 Nobel Peace Prize. Hopefully, the civilized world will continue to recognize this looming threat and will appropriately deal with those who use these instruments of mass destruction.

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Editor's Corner,

About 'Flakka', 'Gravel', or '$5 Insanity' - a new synthetic designer drug

This new drug of abuse has been reported to be on the rise, especially in areas throughout Florida, Ohio, Texas, and Tennessee. It is a synthetic stimulant of the family of cathinones, α-pyrrolidinovalerophenone (α-PVP), originally synthesized in the 1960s, and structurally related to bath salts, such as 3,4-methylenedioxypyrovalerone (MDPV). Categorized as a schedule I controlled substance by the US Drug Enforcement Agency in 2014, α-PVP is most commonly known by its street name of "flakka," Spanish slang for a thin, beautiful woman, or "gravel" because of its similar appearance to white/pink aquarium gravel. This drug can be easily purchased over the Internet from China, India, or Pakistan and it can be eaten, injected, snorted, or vaporized in an e-cigarette. α-PVP inhibits norepinephrine and dopamine transporters preventing their reuptake, and is much more potent than cocaine or amphetamine. The sharp increase in norepinephrine causes many of the physiologic changes, including a dangerously high heart rate and blood pressure; increased dopamine can cause delusions, hallucinations, and increased locomotion. Healthcare providers and law enforcement should be cautious when restraining persons who have used such drugs, because the person will typically vigorously struggle, and consequences can include seizures, arrhythmias, and death. Other effect of these drugs is hyperthermia, which is exacerbated by struggling and agitation. Furthermore, muscle tissue may begin to degrade, leading to rhabdomyolysis. Dehydration may happen due to overexertion. Dehydration and muscle breakdown together can precipitate renal failure.

α-PVP cannot be detected on usual qualitative urine drug testing; however, α-PVP and metabolites can be identified through specific quantitative testing of blood or urine using gas- and liquid-chromatographic–mass spectrometric methods. The treatment of α-PVP intoxication is similar to the treatment of intoxication with bath salts: the use of intravenous benzodiazepines for sedation and seizure prevention until the effects of α-PVP have subsided.

Healthcare professionals should be aware of the newest trends in synthetic drug abuse and the physiologic and psychiatric consequences of intoxication. Patients, especially those with a history of addiction, should be educated about the dangers of substances such as α-PVP. The general population, particularly parents, should take notice to help deter experimentation and subsequent addiction, physical injury, and possible death.

Pradip Datta

Upcoming Conferences

MSACL	Feb 21-25	Palm Springs, CA
PITTCON	March 6-10	Atlanta, GA
KnowledgeLab 2016	March 20-23	Orlando, FL
http://www.midwesttox.org/annualMeeting.html	April 14-15	St Louis, MO
APS	May 11-14	Austin, TX
AACC 2016	July 31-Aug 4	Philadelphia, PA

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