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Optimization of Therapeutic Strategies for Organophosphate Poisoning

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OPTIMIZATION OF THERAPEUTIC STRATEGIES FOR ORGANOPHOSPHATE POISONING

THESIS

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Abstract

Our National Preparedness Vision requires the U.S. to be prepared to prevent, protect against, respond to, and recover from all hazards associated with a chemical attack. Results of this study demonstrate that we cannot protect service members and first responders as required following a nerve agent attack. The research presented herein aimed to construct a physiologically based pharmacokinetic model to determine optimal therapeutic strategies for organophosphate (nerve agent) poisoning. The constructed model integrated organophosphates and two antidotes, atropine and oximes. Currently, both antidotes are fielded to military members of all services for medical treatment. Model results reasonably mirrored literature data and anecdotal observations of organophosphate poisoning. Results suggest a symptoms-based dosing strategy of atropine and a time-based dosing strategy of oximes. For patients severely poisoned with organophosphorus nerve agents, which are to be expected in combat operations, model results support documented claims of oxime's inefficacy and tendency to heighten the severity of poisoning. The results strongly indicate that military personnel attacked with nerve agents are at a significant health risk if they employ their prescribed treatment as current doctrine dictates. Results presented herein suggest that oxime use be discontinued as currently prescribed within the context of nerve agent exposure; its use will not alter the effects of nerve agent exposure and may increase adverse effects.

Acknowledgments

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OPTIMIZATION OF THERAPEUTIC STRATEGIES FOR ORGANOPHOSPHATE POISONING

I. Introduction

Background

In the 1800s, chemists synthesized the first organophosphorus chemical (Szinicz, 2005:173). Researchers later created various forms of the organophosphate and applied the chemicals as insecticides (Szinicz, 2005:173). In the 1930s, a German chemist developed exceptionally lethal organophosphates, which were soon applied to weapon systems and eventually classified as the first nerve agents (Szinicz, 2005:173). Continued investigation of these chemicals over the past seventy years has produced greater variants of organophosphorus insecticides and nerve agents (Cannard, 2006:87).

Organophosphates poison an estimated 100,000 people each year throughout the world (Thiermann and others, 1999:23). The mechanism of poisoning is consistent for all organophosphate varieties (Szinicz and others, 2007:27). When introduced into the body, organophosphates bind to acetylcholine esterases at neural synapses (Cannard, 2006:86). The function of acetylcholine esterases is to hydrolyze neural transmitters, known as acetylcholine molecules (Cannard, 2006:87). Acetylcholine molecules carry neural transmissions across synapses from one nerve cell to another nerve cell (Cannard, 2006:87). While acetylcholine esterases are bound to organophosphates, acetylcholine molecules are not hydrolyzed and are free to repeatedly stimulate the receiving nerve cell (Cannard, 2006:87). This continuous neural stimulation produces the classic symptoms of

organophosphate poisoning, which include, among many, muscle spasms and excess gland secretion (Cannard, 2006:88). The most severe poisonings can result in respiratory failure and death (Cannard, 2006:89).

The two widely accepted antidotes for organophosphate poisoning are atropine and oximes (Cannard, 2006:92). Atropine temporarily binds to neural receptor sites without initiating neural stimulation (Cannard, 2006:92). Atropine's competition for the neural receptor sites reduces acetylcholine molecules' access to these sites and, subsequently, dampens excessive neural stimulation (Cannard, 2006:92). Oximes break organophosphate-acetylcholine esterase bonds (Cannard, 2006:92). Freed acetylcholine esterases may then resume the hydrolysis of acetylcholine molecules and, consequently, also dampen excessive neural stimulation (Cannard, 2006:92).

Despite the wide acceptance of these antidotes to mitigate organophosphate poisoning, various government agencies currently suggest different dosing strategies for the antidotes (Cannard, 2006:91). Some researchers acknowledge that the optimal dose of the antidotes is controversial (Heath and McKeown, 2002:24). The conflicting treatment strategies result from a deficiency in the overall organophosphate research. This research deficiency has inspired the purpose of this thesis.

Research Objectives

1. Construct a physiologically based pharmacokinetic (PBPK) model to predict the tissue concentrations of organophosphates, atropine, oximes, and pertinent biological chemicals.
2. Integrate the reactions among these chemicals into the model and produce a quantitative measurement of their aggregate effects.

3. Apply therapeutic strategies for atropine and oximes against an organophosphate exposure to the model.
4. Compare model output and report significant differences in therapeutic strategies.

Challenges

Over the past fifteen years, a few researchers have applied PBPK modeling to predict levels of organophosphates in human tissue. In 1994, Gearhart and others created the first such PBPK model for two types of organophosphates (Gearhart and others, 1994:1). The researchers provided evidence that a PBPK model for organophosphates could be adapted for cross-species studies and across the family of organophosphorus chemicals (Gearhart and others, 1994:12-13). In 2002, Timchalk and others supported these findings with a similar study on a different organophosphate (Timchalk and others, 2002:42). Also in 2002, Gentry and others conducted a similar study, but the researchers expanded the utility of the PBPK model to incorporate greater interaction between the organophosphates and biological chemicals (Gentry and others, 2002:137). However, none of these studies have fully incorporated the antidotes and their interactions with other chemicals into the model across all tissue groups. As a result, the proposed research will require an expansion of the scope and utility of the models presented in literature.

Research literature provides limited information on model parameters and coefficients for organophosphates and their antidotes. The toxic nature of the chemicals makes human testing difficult and, in some cases, impossible. In addition, Szinicz and others suggest that pharmaceutical companies have little motivation to invest in more detailed research concerning organophosphate poisoning (Szinicz and others, 2007:23-24). Therefore, this research must determine some parameters and coefficients that

produce model results, which mimic limited observations of organophosphate poisoning and antidote efficacy.

Furthermore, some researchers question the efficacy of oximes. Animal and in vitro experiments suggest that oximes are effective, while other reports of organophosphate poisoned humans suggest oximes are ineffective (Szinicz and others, 2007:25). Szinicz and others acknowledge that "the true efficacy of oximes in patients with acute organophosphate poisoning is not known" (Szinicz and others, 2007:25). The researchers suggest that future studies on oximes consider the possibility that acetylcholine esterases, freed from bonds with organophosphates by oximes, rebind with "persisting organophosphates in the body" (Szinicz and others, 2007:26).

Finally, the deleterious effects of organophosphate poisoning are numerous, and the severity of organophosphate poisoning varies across the chemical class (Cannard, 2006:88-89). In addition, the effectiveness of an antidote dose varies with the severity of organophosphate toxicity (Cannard, 2006:91). This study must aggregate the reactions among organophosphates, antidotes, and biological chemicals to produce a single, quantifiable result in order to facilitate comparison among different therapeutic strategies.

Justification and Applicability

Organophosphorus insecticides are one of the most predominant insecticides used today (Reigart and Roberts, 1999:34). Over forty registered brands of insecticides contain organophosphorus chemicals (Reigart and Roberts, 1999:34). In 1996, the United States accounted for more than 4,000 of the yearly organophosphate poisonings experienced throughout the world (Reigart and Roberts, 1999:5). Since relatively minor poisonings do not necessarily initiate medical treatment, these numbers are likely an underestimate of

actual organophosphate poisonings. Most organophosphate poisonings occur through association with the agricultural industry by accidental and excessive exposure or by improper application of insecticides (Calvert and others, 2004:20).

Sarin, soman, tabun, and VX are the most common nerve agents (Cannard, 2006:87). Despite being first developed in the 1930s, it was not until the 1980s, well after international bodies established protocols to curb use of these chemicals, that the first employment of nerve agents as a weapon was documented (Szinicz, 2005:172). Iraq employed tabun and sarin against Iranian military forces between 1983 and 1984 and between 1987 and 1988 during the Iran-Iraq War (Szinicz, 2005:172). Iraq also employed sarin against civilian Iraqi Kurds, an ethnic sub-population of Iraq, between 1987 and 1988 (Szinicz, 2005:172).

In 1994, Aum Supreme Truth, a religious cult, synthesized sarin and employed the chemical against Japanese government agencies and citizens to further the cult's political and religious goals (Yanagisawa and others, 2006:76). The terrorist cult released 12 liters of a 70% sarin solution in Matsumoto, Japan (Yanagisawa and others, 2006:77). The nerve agent killed 7 people and caused 56 hospital inpatient casualties, 208 hospital outpatient casualties, and 277 on-scene treated sicknesses (Yanagisawa and others, 2006:77). In 1995, the terrorist group again released sarin, this time on the Tokyo subway, killing 12 people and causing over 500 illnesses, which included 100 first care responders. (Yanagisawa and others, 2006:81)

The history of organophosphates is disturbing. Despite growing knowledge of the toxicity of these chemicals and government controls and education efforts to reduce poisonings, the use of these chemicals and number of annual poisonings remains

significantly high. In addition, despite international efforts to control the development, storage, proliferation, and use of nerve agents, it has not been enough to deter governments and terrorist organizations from creating and employing these chemicals.

The threat of organophosphate poisoning is genuine and current. A more complete understanding of organophosphate antidotes could help to more effectively mitigate symptoms and save lives. The rapid onset of organophosphate induced symptoms and the chemicals' potential to quickly cause death, especially from the more toxic organophosphates, provides motivation to more accurately and decisively define the dosing strategies for prompt and proper treatment.

II. Literature Review

History of Methodology

Since the development of organophosphates, researchers have conducted much study to better understand the chemicals. In the late 1980s, a subset of the overall organophosphate research arose that involves the application of physiologically based pharmacokinetic (PBPK) modeling (Maxwell and others, 1987:66). PBPK models predict tissue dose concentrations of chemicals in organisms with respect to time (Andersen, 2003:10). The model categorizes human mass into discrete tissue groups with similar pharmacokinetic properties (Andersen, 2003:12). Modelers create mass balance differential equations around each tissue group (Andersen, 2003:11). Equation parameters include tissue volumes, blood flow rate, breathing rate, metabolic constants, and unique chemical characteristics (Andersen, 2003:12). The modeling tool numerically integrates the equations to determine the chemical amounts in the tissue over time (Andersen, 2003:11).

PBPK modeling is predominantly used to predict human tissue concentrations of a chemical by inferring data from laboratory experiments conducted on animals (Andersen, 2003:13-14). In general, this method gains data from test animals, applies the data to a PBPK model, adjusts the model to simulate experimental results, and then applies human parameters to the model in place of animal parameters to predict human results (Andersen, 2003:13-14). Modelers then repeatedly execute the human model over a wide range of scenarios that were not studied in the laboratory experiment (Andersen,

2003:10). Not only does this technique save time and money, but it also allows researchers to obtain human results when human experiments are impossible or severely limited due to the toxicity of the chemical (Andersen, 2003:14). In addition, the modeling technique allows researchers to obtain results for humans exposed to low dose concentrations of a chemical over decades from data obtained through laboratory experiments on animals exposed to high dose concentrations of a chemical over days or weeks (Andersen, 2003:10).

Another valuable application of PBPK modeling is to create a hypothesis for a future laboratory experiment based on the results of the model, which was created from the current understanding of physiology and the chemicals under study (Andersen, 2003:14). This technique is particularly useful when physiological mechanisms and chemical interactions are vaguely understood or are unproved and when historical data are limited. A successful model will predict future results of laboratory experiments and help provide evidence to support understood mechanisms (Andersen, 2003:14). However, and ironically, an unsuccessful model, which fails to predict future laboratory results, is likely more useful in that it initiates rigorous questioning of understood mechanisms. This rigorous questioning may lead to insights and greater understanding of a chemical and its interactions with physiology (Andersen, 2003:14).

The application of PBPK modeling for organophosphates began in 1988 with a study by Maxwell and others (Maxwell and others, 1988:66). The researchers dosed rats with 90 μg/kg of soman and measured tissue concentrations of the organophosphate and acetylcholine esterase inhibition (Maxwell and others, 1988:68). These measurements were used to determine any correlation among the organophosphate tissue concentration,

esterase inhibition, and physiological parameters (Maxwell and others, 1988:67). Maxwell and others found a strong correlation between esterase inhibition and blood flow, a key component of PBPK modeling (Maxwell and others, 1988:69-72). As a result, the researchers suggested “that it may be possible to use a flow-limited physiological pharmacokinetic model to describe the kinetics of in vivo esterase inhibition by soman” (Maxwell and others, 1988:66).

In 1994, Gearhart and others tested Maxwell’s suggestion. The researchers determined “to develop a quantitative, physiologically based model for organophosphate pharmacokinetics and acetylcholine esterase inhibition” (Gearhart and others, 1994:3). The researchers dosed rats with the organophosphate, diisopropylfluorophosphate (DFP), and measured tissue concentrations of the organophosphate and esterase activity (Gearhart and others, 1994:5). The researchers then constructed a model and optimized parameters until the model simulated the experimental data (Gearhart and others, 1994:5-6). Next, the researchers applied human parameters to the model (Gearhart and others, 1994:5-6). Model results for the human reasonably mirrored DFP data obtained from literature concerning DFP therapeutic treatments (Gearhart and others, 1994:13). Furthermore, the researchers then adapted the model to another organophosphate, parathion (Gearhart and others, 1994:13). Again, the model results reasonably mirrored literature data (Gearhart and others, 1994:13). The researchers provided evidence that the PBPK model could predict tissue concentrations of organophosphates, that the model was applicable for cross-species studies, and that the model was applicable across the family of organophosphorus chemicals (Gearhart and others, 1994:12-13).

In 2002, Timchalk and others created a similar PBPK model for chlorpyrifos (CFP), a less lethal organophosphate, which is used in insecticides (Timchalk and others, 2002:34). The researchers fed rats with CFP and measured tissue concentrations of CFP over time (Timchalk and others, 2002:35). Using the experimental data, literature data, and optimization techniques, the researchers successfully constructed a model that produced results that mirrored the experiment (Timchalk and others, 2002:35). The researchers then applied human parameters to the model (Timchalk and others, 2002:41). As with the study by Gearhart and others, the model reasonably mirrored experimental data obtained from human testing from another study (Timchalk and others, 2002:42). Again, more evidence supported PBPK modeling of organophosphates (Timchalk and others, 2002:42).

In 2002, Gentry and others created a similar PBPK model for the organophosphate, parathion, and its metabolite, paraoxon (Gentry and others, 2002:120). The researchers intended to provide an estimate on how polymorphism affects tissue doses and toxicity of a chemical (Gentry and others, 2002:120). Although the results of the experiment showed that the genetic variations had little effect on the toxicity of parathion, the study did further provide evidence for applying PBPK modeling to determine tissue concentrations of organophosphates (Gentry and others, 2002:131). In addition, the researchers were able to expand the utility of the model by incorporating greater interaction between the organophosphate and biological chemicals (Gentry and others, 2002:125-137).

PBPK modeling is highly dependent upon the mechanisms of the cardiovascular system. Organophosphates and their antidotes interact extensively with the nervous

system. To facilitate a more detailed explanation of the mechanisms of the chemicals and PBPK modeling, the next two sections will provide a brief review of the cardiovascular and nervous systems.

Cardiovascular System

The cardiovascular system consists of the heart and a network of closed loop blood vessels (Fox, 2006:382). Arteries transport blood away from the heart and veins return blood to the heart (Fox, 2006:382). Capillaries are smaller and more numerous vessels that connect arteries and veins in tissue (Fox, 2006:406).

Blood consists of erythrocytes, leukocytes, and platelets, which are suspended in plasma (Fox, 2006:384-385). Plasma is predominantly water and solutes (Fox, 2006:383).

An important function of the cardiovascular system is to carry nutrients, waste, and other chemicals to and from tissue (Fox, 2006:382). All transfer of materials to and from the blood and tissue occurs across capillary walls (Fox, 2006:382-383). The human body consists of more than 40 billion capillaries that provide nearly 1000 square miles of surface area for diffusion of chemicals between blood and tissue (Fox, 2006:408). Nearly every cell in the human body is within 60 to 80 μm of a capillary (Fox, 2006:408).

Blood pressure drives water and solutes from the plasma through capillary walls into interstitial fluid at the arterial end of capillaries (Fox, 2006:431). As blood pressure drops at the venous end of the capillaries, concentration gradients will drive some amounts of water and solutes from the interstitial fluid back into the capillaries (Fox, 2006:432).

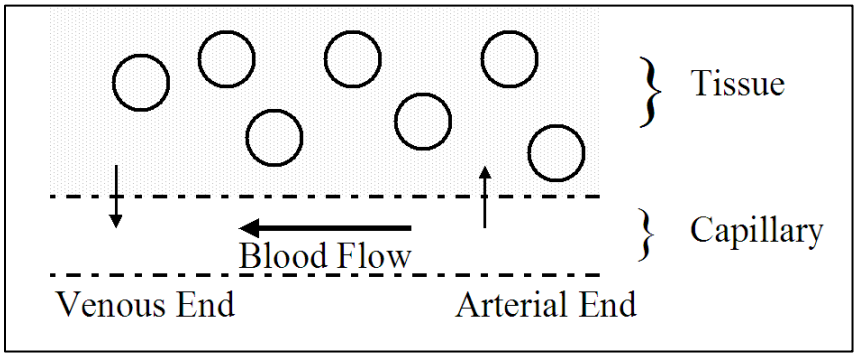

Figure 1. Capillary Diffusion (Fox, 2006:431-432)

Nervous System

Motor neurons are classified as somatic or autonomic (Fox, 2006:156). Somatic motor neurons direct the reflexive and voluntary control of skeletal muscles (Fox, 2006:156). Originating in the central nervous system (CNS), somatic neurons extend into the peripheral nervous system (PNS) and terminate at receptors of skeletal muscles (Fox, 2006:156). Autonomic motor neurons are located in the PNS (Fox, 2006:156). Autonomic neurons receive neural transmission from other neurons extending from the CNS and relay the neural transmission to receptors of involuntary tissues: smooth muscles, cardiac muscle, and glands (Fox, 2006:156).

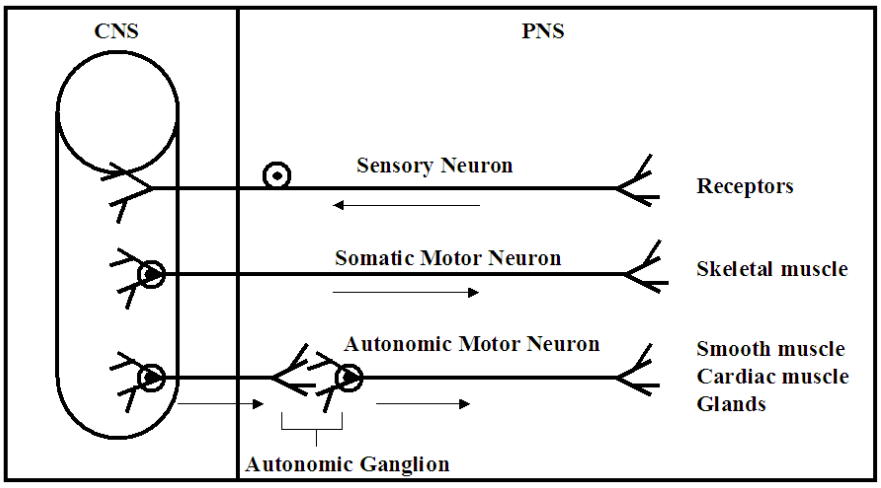

Figure 2. Central and Peripheral Nervous System (adapted from Fox, 2006:156)

An approximately 10 nm wide cleft, called a synapse, separates neurons from other neurons, muscles, and glands (Fox, 2006:172). Adhesion molecules that project from both sides of the synapse hold the width of the cleft constant. (Fox, 2006:171) Neural transmission across synapses of motor neurons is one-way, in the direction from the CNS to the receptor, and occurs with the release of neurotransmitters from the pre-synaptic neuron and the reception of neurotransmitters by the post-synaptic cell (Fox, 2006:172). Acetylcholine molecules are the most common neurotransmitters in the body and the ones indirectly affected by organophosphate poisoning (Fox, 2006:175).

The pre-synaptic neuron contains small sacs, which store acetylcholine molecules (Fox, 2006:172). Upon neural stimulation, the sacs fuse with the membrane of the pre-synaptic neuron and create pores through which the acetylcholine molecules diffuse into the synaptic cleft (Fox, 2006:172). The amount of acetylcholine molecules released into the cleft is dependent on the amount of acetylcholine molecules in the sac, the number of sacs fusing to the membrane, and the frequency of neural transmission (Fox, 2006:172).

Some of the sacs are pre-staged at the pre-synaptic nerve cell membrane to increase the speed of neural transmission (Fox, 2006:172).

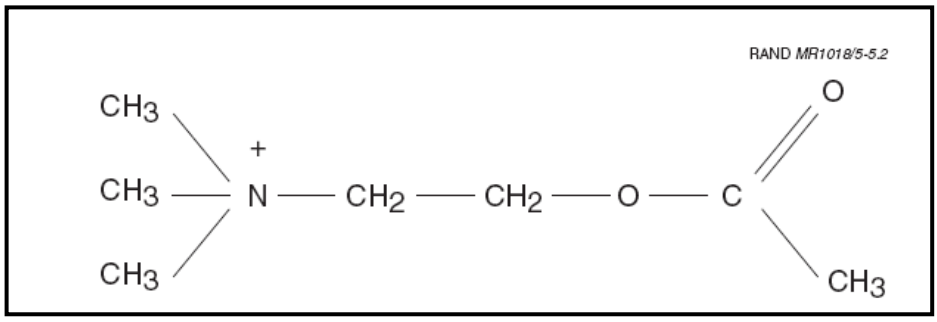

Figure 3. Acetylcholine Structure (Rand, 2007:116)

Once in the cleft, acetylcholine molecules diffuse across the synapse through interstitial fluid and briefly bind to receptor sites on the post-synaptic cell (Fox, 2006:173-177). It is these binds between acetylcholine molecules and the post-synaptic receptor sites that stimulate the neural functioning of the post-synaptic cell (Fox, 2006:173). After a short time, acetylcholine molecules will disassociate from the receptor sites and maintain the potential to re-bind to the receptors (Fox, 2006:177).

There are two types of cholinergic receptors that receive acetylcholine molecules: nicotinic receptors and muscarinic receptors (Fox, 2006:174-175). Nicotinic receptors are found between neurons and skeletal muscles, between neurons and non-voluntary muscles, and between some neurons and other neurons in the CNS (Fox, 2006:174). Muscarinic receptors are found between neurons and glands in the PNS and also between some neurons and other neurons in the CNS. (Fox, 2006:175)

Acetylcholine esterases are enzymes embedded on the post-synaptic cell and which terminate the action of acetylcholine molecules (Fox, 2006:177). The serine hydroxyl group of the acetylcholine esterase binds to the acetyl portion of the acetylcholine. With this bind, the choline moiety of the acetylcholine is released

(Cannard, 2006:87). Hydrolysis then separates the acetyl moiety from the acetylcholine esterase (Cannard, 2006:87). The choline moiety will return to the pre-synaptic cell to be recycled for the creation of new acetylcholine, while the acetyl group will react with water to form acetic acid (Cannard, 2006:87). Acetylcholine esterases are extremely effective and each enzyme has the potential to hydrolyze 300,000 acetylcholine molecules per minute (Hoskins and Ho, 1992:289). Acetylcholine esterases are the only enzymes that hydrolyze acetylcholine. Without acetylcholine esterases, acetylcholine molecules will persist in the synaptic cleft, continually bind and disassociate with receptor sites, and cause excessive neural stimulation (Fox, 2006:178).

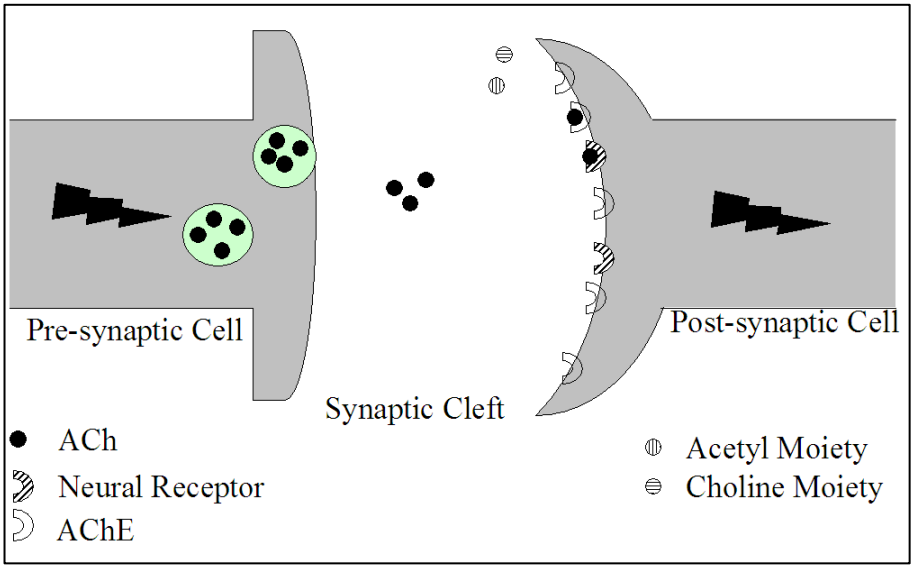

Figure 4. Synaptic Cleft (adapted from Fox, 2006:178)

Organophosphates

Organophosphates are liquid chemicals (Cannard, 2006:87). An organophosphate is characterized by central phosphorous atom bound to an oxygen atom, two alkyl groups, and a leaving group (Cannard, 2006:87). Although liquid, organophosphates are, generally, easily volatized (Cannard, 2006:87). As a result, the most likely human exposure to organophosphates is through inhalation, although intake of the chemicals through dermal absorption or ingestion is possible (Cannard, 2006:87).

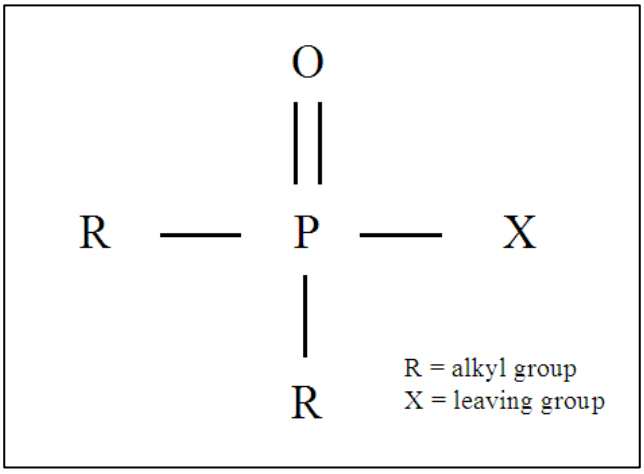

Figure 5. Organophosphate Structure (Cannard, 2006:87)

Regardless of the entry route into the body, organophosphates will diffuse into the blood stream, and the cardiovascular system will transport the chemicals to all tissue groups. In the tissue groups, organophosphates will move towards neural synapses and bind to acetylcholine esterases (Cannard, 2006:87). After a period of time, which is different for each organophosphate type, the organophosphate-esterase bonds will mature by the de-alkylation of the organophosphates (Cannard, 2006:87). Upon maturation, the

organophosphate-esterase bonds will become irreversible and both the organophosphates and acetylcholine esterases are destroyed. (Cannard, 2006:87)

When acetylcholine esterases are bound to organophosphates, acetylcholine molecules are not hydrolyzed, accumulate in the synapse, and cause over-stimulation of the nervous system (Cannard, 2006:87). This over-stimulation leads to a variety of physiological effects, which are dependent on the type of neural receptor and location in the body (Cannard, 2006:88-89).

In the PNS, over stimulation of muscarinic receptors causes continuous contraction of smooth muscles and secretion of exocrine glands (Cannard, 2006:88). Effects include "miosis with dim or blurred vision, eye pain (ciliary spasm) or headache, tearing, rhinorrhea, salivation, bronchoconstriction and excessive bronchosecretions with dyspnea, bradyarrhythmias, hypotension, nausea and vomiting, abdominal cramps, diarrhea and bowel incontinence, and urinary incontinence" (Cannard, 2006:88). Also in the PNS, organophosphates lead to the over-stimulation of nicotinic receptors causing uncontrolled contraction of voluntary muscles (Cannard, 2006:87).

Both muscarinic and nicotinic receptors are found in the CNS (Fox, 2006:156). In the CNS, over-stimulation of both receptor types causes "mild to severe behavioral and cognitive changes, impaired consciousness or coma, seizures, or central apnea" (Cannard, 2006:89). If the exposed person survives the initial effects of organophosphate poisoning, other symptoms may persist for weeks and include "irritability, anxiety, depression, fatigue, insomnia, nightmares, and impaired judgment" (Cannard, 2006:89).

The only way to terminate the effects of organophosphate poisoning is the elimination of the organophosphates from the body and the full recovery of acetylcholine

esterases (Cannard, 2006:87). Destruction of the organophosphates is relatively quick via natural metabolic degradation. However, the recovery of acetylcholine esterases is relatively slow (Cannard, 2006:87). Effects from organophosphate poisoning can persist for weeks as a result of deficient acetylcholine esterases in tissue groups and not from organophosphate persistence in the body (Cannard, 2006:89).

Four naturally occurring chemicals in the body will destroy organophosphates (Gearhart and others, 1994:4). At the neural synapse, acetylcholine esterases initiate the release of the organophosphates' leaving group and the destruction of the organophosphates upon bond maturation (Cannard, 2006:87).

In addition to being found in tissue groups, butyrylcholinesterases and other acetylcholine esterases are found in the blood stream and have the first opportunity to attach to organophosphates (Cannard, 2006:86). Like organophosphate-acetylcholine esterase bonds, organophosphate-butyrylcholinesterase bonds become irreversible with maturation, and the butyrylcholinesterases and organophosphates are destroyed with the separation of the organophosphates' leaving group (Gearhart and others, 1994:4). Unlike acetylcholine esterases, the loss of butyrylcholinesterases to organophosphate poisoning appears to have no imminent effect on life sustaining functions (Cannard, 2006:88).

Carboxylesterases are found throughout the body and in much greater numbers than acetylcholine esterases and butyrylcholinesterases. These enzymes also bind to and mature with organophosphates (Gearhart and others, 1994:4). As with butyrylcholinesterases, there is no known adverse physiological affect from the loss of carboxylesterases.

The enzyme suite, cytochrome P450, metabolizes organophosphates. (Levi and Hodgson, 1992:142). Elements of this enzyme suite are found in all tissue groups except fat tissue (Gearhart and others, 1994:3).

Antidotes

There are three widely accepted medications to treat organophosphate poisoning. The first medication, oxime, is introduced into the body intravenously or intramuscularly (Cannard, 2006:92). Oximes will attack the organophosphate-esterase bonds and separate the chemicals (Cannard, 2006:92). However, oximes are only effective prior to maturation of the organophosphate-esterase bonds (Cannard, 2006:92). For reference, the maturation half-time of soman is 2-6 minutes, while the maturation half-time for tabun, sarin, and VX is between 5 and 48 hours (Cannard, 2006:92). Esterases are fully recovered and operational with effective separation from the organophosphates (Cannard, 2006:92). However, oximes appear to "have little effect on muscarinic symptoms and signs" (Cannard, 2006:92). There are a few varieties of oximes, but they all operate in essentially the same manner (Cannard, 2006:92). The U.S. uses Pralidoxime Chloride (2-Pam Cl) (Cannard, 2006:92).

Atropine may also be introduced into the body intravenously or intramuscularly (Cannard, 2006:92). At muscarinic receptors only, atropine will repeatedly bind to and dissociate with the neural receptors without causing neural stimulation (Cannard, 2006:92). The presence of atropine will reduce the availability of muscarinic receptors to acetylcholine molecules (Cannard, 2006:92). As a result, atropine will reduce the excessive secretion of exocrine glands and the over-stimulation of smooth muscles (Cannard, 2006:92). The body will naturally metabolize or excrete atropine over time.

At high doses, atropine can cause adverse health effects (USAMRICD, 2000:120). Generally, people not exposed to organophosphates with doses of 10 mg of atropine or higher may experience delirium (USAMRICD, 2000:120). In addition, the blockage of neural receptors at glands may inhibit sweating and endanger an individual to heat related injuries (USAMRICD, 2000:120). Furthermore, small amounts of atropine can cause blurred vision for up to a day (USAMRICD, 2000:123).

The third medication for organophosphate poisoning is an anticonvulsant, and diazepam is the preferred medicine (Cannard, 2006:92). Diazepam reduces the severity of seizures and epilepsy, which are caused by over-stimulation of muscarinic and nicotinic receptors in the CNS (Cannard, 2006:92-93). This medication will not be studied in the model.

Therapeutic Strategies

Therapeutic strategies vary among government agencies (Cannard, 2006:91). The Centers for Disease Control and Prevention (CDC) base initial dosing procedures on observations of symptoms (CDC, 2008:13). The symptoms are classified as either mild-moderate or severe (CDC, 2008:13). "Mild-moderate symptoms include localized sweating, muscle fasciculations, nausea, vomiting, weakness, and dyspnea" (CDC, 2008:13). For adults with these symptoms, first care responders are directed to initially administer one or two atropine injections of 2 mg each and one 2-Pam Cl injection of 600 mg (CDC, 2008:13). Additional atropine doses are repeated every 5 to 10 minutes (CDC, 2008:13). There are no instructions to first care responders for repeated doses of 2-Pam Cl. Both atropine and 2-Pam Cl injections are administered intramuscularly by first care responders (CDC, 2008:13).

"Severe symptoms include unconsciousness, convulsions, apnea, and flaccid paralysis" (CDC, 2008:13). For adults, first care responders are directed to administer three atropine injections of 2 mg each (CDC, 2008:13). Additional atropine injections of 2 mg should be administered "at 5 to 10 minute intervals until secretions have diminished and breathing is comfortable or airway resistance has returned to normal" (CDC, 2008:13). Eighteen hundred mg of 2-Pam Cl are administered with the initial atropine injection. (CDC, 2008:13). As with mild to moderate symptoms, there are no instructions for additional doses of 2-Pam Cl (CDC, 2008:13).

Medical doctors with appropriate equipment are provided slightly different instructions (CDC, 2008:19). The procedure for administering atropine is identical to the instructions provided to first care responders (CDC, 2008:19). However, medical doctors will slowly administer 15 mg/kg of 2-PAM Cl intravenously for mild-moderate and severe symptoms (CDC, 2008:19). In addition, medical doctors may administer 5 mg of diazepam intravenously for patients with convulsions (CDC, 2008:19).

The New York Department of Health (NYDH) provides slightly different procedures for first care responders (NYDH, 2008:4). For mild to moderate symptoms, 2 to 4 mg of atropine and 600 mg of oxime are administered intramuscularly (NYDH, 2008:4). For severe symptoms, 6 mg of atropine and 1800 mg of oxime are administered intramuscularly (NYDH, 2008:4). For all cases, unspecified amounts of atropine are repeated every 2 to 5 minutes until breathing has returned to near normal (NYDH 2008:4). Unspecified amounts of 2-Pam Cl are repeated once between 30 to 60 minutes and then 1 to 2 doses every hour thereafter (NYDH, 2008:4).

Yet again, the U.S. Army Medical Research Institute of Chemical Defense (USAMRICD) provides slightly different instructions (USAMRICD, 2008:123-125). For mild symptoms, 2 mg of atropine and 600 mg of 2-Pam Cl are administered (USAMRICD, 2008:123-124). For severe symptoms, 6 mg of atropine and 1800 mg of 2-Pam Cl are administered (USAMRICD, 2008:124). Additional doses of 2 mg of atropine are repeated every three to five minutes until breathing is near normal (USAMRICD, 2008:123-124). There are no instructions for administering additional doses of 2-Pam Cl (USAMRICD, 2008:124).

Finding the correct dosage of atropine is difficult (USAMRICD, 2008:124). There is a risk of giving too much atropine to a patient experiencing mild symptoms, and therefore, produce adverse effects on the patient from the atropine (USAMRICD, 2008:124). In addition, there is a risk of giving too little atropine to a patient with severe symptoms, and experience the risk of not properly treating the patient (USAMRICD, 2008:124).

Table 1. Therapeutic Strategies for Mild to Moderate Symptoms

		CDC	NYPH	USAMRICD
Atropine	Initial Dose	2 – 4 mg	2 – 4 mg	2 mg
	Repeat Dose	2 mg	Unspecified	2 mg
	Repeat Interval	5 – 10 min	2 – 5 min	3 – 5 min
2-Pam Cl	Initial Dose	600 mg	600 mg	600 mg
	Repeat Dose	No Instructions	600 – 1200 mg	No Instructions
	Repeat Interval	No Instructions	Once b/w 30 – 60 min & every hr thereafter	No Instructions

(CDC, 2008:13; NYDH, 2008:4; USAMRICD 2008:124)

Table 2. Therapeutic Strategies for Severe Symptoms

		CDC	NYPH	USAMRICD
Atropine	Initial Dose	6 mg	6 mg	6 mg
	Repeat Dose	2 mg	Unspecified	2 mg
	Repeat Interval	5 –10 min	2 – 5 min	3 – 5 min
2-Pam Cl	Initial Dose	1800 mg	1800 mg	1800 mg
	Repeat Dose	No Instructions	600 – 1200 mg	No Instructions
	Repeat Interval	No Instructions	Once b/w 30 –60 min & every hr thereafter	No Instructions

(CDC, 2008:13, NYDH, 2008:4, USAMRICD 2008:124)

Physiologically Based Pharmacokinetic Modeling

PPBK modeling describes the tissue-dose concentrations of a chemical with respect to time (Andersen, 2003:10). A mass balance concept is applied to the model to describe absorption, distribution, metabolism and excretion of chemicals (Hoang, 2003:99). Absorption, or entry, of a chemical into the model is primarily described by inhalation, dermal absorption, or ingestion (Hoang, 1995:101). Chemical excretion from the model occurs by metabolizing the chemical into an irrelevant metabolite, loss of the chemical in whole with urine, or volatilization of the chemical from the blood into the lungs (Hoang, 1995:102).

Within the model, the entire mass of the organism under study is grouped into discrete tissue compartments with similar physiological and pharmacokinetic properties (Hoang, 1995:101). Mass balance equations are created for each compartment to describe the concentration of the chemical in those compartments with respect to time (Hoang, 1995:101).

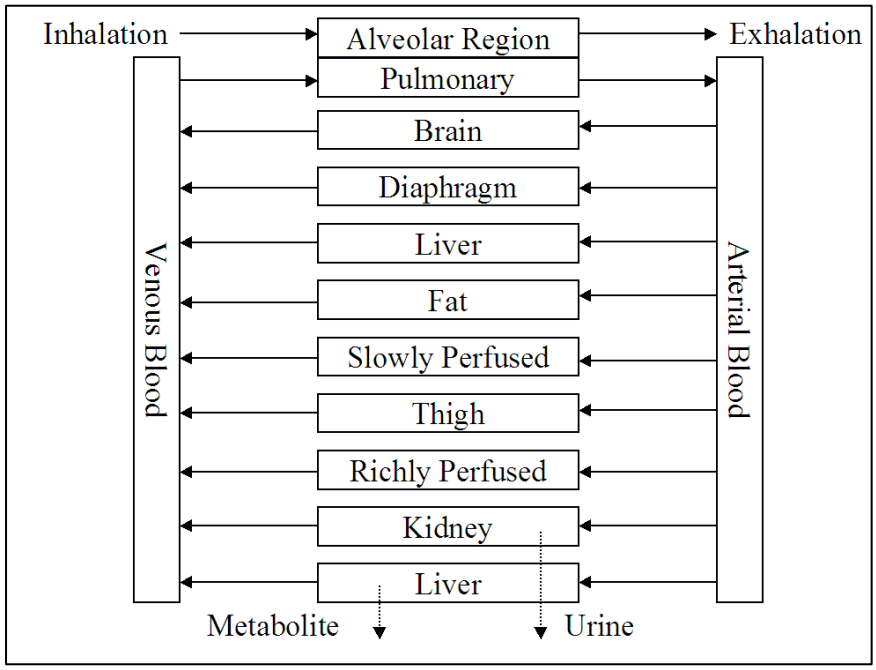

Figure 6. Basic PBPK Schematic (Gearhart and others, 1994:4)

Distribution of the chemical throughout the model occurs via the blood flow. The product of the fraction of blood flowing into each compartment, the concentration of the chemical in the blood (mass/volume), and the cardiac output (volume/time) determines the amount of chemical entering the compartment (mass/time).

Absorption of the chemical from the blood into the tissue compartment assumes a lumped-parameter approach (Hoang, 1995:101). In this assumption, the chemical completely diffuses from the blood into the tissue compartment and instantaneously achieves a homogenous and well-mixed state throughout the interstitial fluid of that compartment (Hoang, 1995:101).

A partition coefficient is used to describe the diffusion of the chemical from the tissue compartment into the venous blood flow. The amount of chemical leaving a

compartment (mass/time) is equal to the product of the fraction of blood flow from the compartment, the cardiac output (volume/time), the concentration of the chemical in the tissue compartment (mass/volume), and the inverse of the partition coefficient. A higher partition coefficient causes a slower outflow of the chemical from the compartment as compared to a lower partition coefficient.

Within each compartment, binding and bio-transformation of the chemical will affect the net accumulation rate of the chemical (Hoang, 1995:101). For example, mathematical equations are created to describe the alteration of chemicals to irrelevant byproducts through reaction with other chemicals and enzymes (Hoang, 1995:101).

PBPK modeling relies on pharmacokinetic data from laboratory experiments (Hoang, 1995:100). Typically, laboratory data are not complete (Hoang, 1995:100). Unknown parameters and metabolic constants are often determined or estimated through model fitting (Hoang, 1995:100).

Chemical Reactions

One of the common chemical reactions that occur in the compartments of PBPK models is the metabolism of a chemical by an enzyme to an irrelevant metabolite (Hoang, 1995:102). This chemical reaction is typically described as follows.

$$\text{Enzyme} + \text{Substrate} \underset{k_2}{\overset{k_1}{<->}} \text{Enzyme-Substrate Complex} \xrightarrow{k_3} \text{Enzyme} + \text{Product}$$

The chemical reaction can be mathematically expressed according to equation (1) (Clark, 1996:446).

$$\begin{aligned} d[\text{Enzyme-Substrate Complex}]/dt = {} & k1[\text{Enzyme}][\text{Substrate}] \\ & - k2[\text{Enzyme-Substrate Complex}] \\ & - k3[\text{Enzyme-Substrate Complex}] \end{aligned} \quad (1)$$

Equation (1) is usually simplified to equation (2) (Clark, 1996:446-448).

d[Enzyme-Substrate Complex]/dt = (Vmax[Substrate])/(Km + [Substrate] (2)

Equation (2) is particularly useful since the coefficients, Vmax and Km, for this formula are found for many chemicals in literature. It is important to note that the enzyme is not destroyed in this reaction. This chemical reaction is applicable to the cytochrome P450 metabolism of organophosphates, atropine, and oximes. For reactions between organophosphates and esterases, the chemical reaction and equation (2) are not applicable. Esterases are destroyed with bond maturation. Hence the chemical reaction between organophosphates and esterase is more accurately expressed as follows.

$$\text{Enzyme} + \text{Substrate} \underset{k_2}{\overset{k_1}{<->}} \text{Enzyme-Substrate Complex} \xrightarrow{k_3} \text{Aged Enzyme-Substrate}$$

The chemical reaction is mathematically expressed according to equation (1) and cannot be simplified.

Literature Data

Gearhart and others first applied PBPK modeling to organophosphates (Gearhart and others, 1994:1). Much of the basic model structure and data were incorporated into many of the organophosphate PBPK models found in literature that followed. In their study, Gearhart and others provided human physiological data such as body weight, ventilation rate, cardiac output, organ volumes, and fraction of blood flows to tissue groups (Gearhart and others, 1994:5). In addition, the researchers provided the partition coefficients and metabolic constants for the organophosphate, DFP (Gearhart and others, 1994:3-6). These values are listed in Appendix B.

Gentry and others investigated the effects of polymorphisms on the metabolism of parathion (Gentry and others, 2002:120). To conduct their study, the researchers needed to incorporate the kinetics between organophosphates and esterases into their model (Gentry and others, 2002:127-128). Initial concentration, synthesis constants, and degradation constants for the esterases were obtained from literature data, experiment, and model fitting. The constants were useful for this study and are listed in Appendix B.

In 2004, Ashani and Pistinner constructed a PBPK model to determine the use of exogenous butyrylcholinesterases as a prophylactic to protect against organophosphate poisoning (Ashani and Pistinner, 2004:358). Their model was based on the concept that free butyrylcholinesterases in the blood stream had the first opportunity to react with organophosphates (Ashani and Pistinner, 2004:359). If enough butyrylcholinesterases were present in the blood stream, then much of the organophosphate would react with butyrylcholinesterases before the organophosphate could enter tissue groups and destroy acetylcholine esterases in those tissue groups (Ashani and Pistinner, 2004:359). Furthermore, because butyrylcholinesterases have a higher affinity for organophosphates as compared to acetylcholine esterases, exogenous butyrylcholinesterases would additionally protect some levels of acetylcholine esterases in the blood (Ashani and Pistinner, 2004:365). In their study, the researchers showed that between 48% and 68% of organophosphates were degraded in plasma before they ever reacted with esterases in tissue (Ashani and Pistinner, 2004:364). In addition, Ashani and Pistinner postulated with other researchers that acetylcholine esterase inhibition in tissue "should be well above 65% to produce visible signs" of symptoms and that enzyme inhibition down to 10% of

basal levels may still permit critical physiological functions (Ashani and Pistinner, 2004:365).

In 1985, Hinderling and others conducted a study to determine the pharmacokinetics of atropine (Hinderling and others, 1985:703). In two separate tests, the researchers intravenously dosed three human males with 1.35 and 2.15 mg of atropine (Hinderling and others, 1985:704). Plasma and urine levels of atropine and the atropine's metabolite, tropine, were measured with a gas chromatographic mass spectrometry (Hinderling and others, 1985:703). The researchers determined that 57% of atropine was not metabolized and eliminated in whole in the urine (Hinderling and others, 1985:703).

A report for the International Program on Chemical Safety Evaluation in 2002 provides a summary of atropine studies (Heath and McKeown, 2007:1). The authors, Heath and McKeown, acknowledged that there is little understanding of the "optimal dose and pharmacokinetics of atropine in relation to the doses of oximes, the severity of (organophosphate) poisoning, and the properties of a particular organophosphate" (Heath and McKeown, 2007:30). In their report, the authors cited a study by Schoene and others that showed pre-dosing rats with atropine before the onset of organophosphate exposure reduced morality rates for the rats (Heath and McKeown, 2007:15). The authors cited another study by Matsubara and Horikoshi that suggested atropine alone was more effective than 2-Pam Cl in treating organophosphate poisoning (Heath and McKeown, 2007:17). The authors further cited an additional experiment by Gupta, who dosed buffalo calves with an organophosphate, atropine, and 2-Pam Cl (Heath and McKeown, 2007:17). Gupta suggested that a therapeutic strategy of atropine and 2-Pam Cl in

conjunction was more effective than either antidote working alone (Heath and McKeown, 2007:17).

Heath and McKeown also reported on a study that stated atropine reaches a peak level in plasma at about 30 minutes after the administration of atropine (Heath and McKeown, 2007:22). Another study reported that only 5% of atropine remains in the blood after 5 minutes following an intravenous injection (Heath and McKeown, 2007:23). A third study showed that atropine's "initial distribution half-life is approximately one minute." (Heath and McKeown, 2007:23).

Heath and McKeown point out the complications and difficulties in determining the optimal dose of atropine for organophosphate poisoning (Heath and McKeown, 2007:24). Numerous case studies show the need for atropine doses ranging from 2 mg to as high as 50 mg, and some doses as much as 30,000 mg, over a period of 3 weeks (Heath and McKeown, 2007:24-25). Ultimately, the amount of atropine is determined by the observation of organophosphate induced symptoms (Heath and McKeown, 2007:26).

Little data is available for oximes and the antidote's efficacy is in question. Szinicz and others reviewed and reported some of the complications of this antidote in a 2007 paper (Szinicz and others, 2007:23). The researchers cited studies that confirm the efficacy of oximes for "in vitro and animal experiments" (Szinicz and others, 2007:25) However, "human reports of severe toxicity frequently mention the failure of oxime therapy and conclude oximes to be ineffective, or even harmful." One of the suggestions for the failure of the oximes is that there is "re-inhibition of the reactivated enzyme by persisting organophosphates" in the body (Szinicz and others, 2007:26). The researchers suggest that future studies consider the total net effect of organophosphates and oximes

and consider “the various interactions between inhibitor, reactivator, and acetylcholine esterases” (Szinicz and others, 2007:26).

In a 1999 article, Thiermann and others presented and discussed the numerous chemical interactions among organophosphates, oximes, and enzymes (Thiermann and others, 1999:234). The researchers suggested that these chemical reactions can be expressed mathematically with reaction rate constants (Thiermann and others, 1999:234).

In 2007, Bartling, Worek, Szinicz, and Thiermann investigated the reactions between organophosphates and esterases (Bartling and others 2007:166). In their article the researchers provided chemical kinetic rate constants for several organophosphates, which proved useful for the work presented in this thesis (Bartling and others, 2007:169).

III. Methodology

Modeling Tool

Model construction and numerical integration was performed with the computer software, STELLA, version 8.0, developed by High Performance Systems Inc.

Model Structure

The model structure describing absorption, distribution, metabolism, and excretion was heavily based on physiological mechanisms and the model developed by Gearhart and others (Gearhart et. al. 1994:4). The basic model structure is depicted in Figure 7. The model neglected volume and mass of pulmonary tissue; however, a pulmonary compartment was created to describe pulmonary functions.

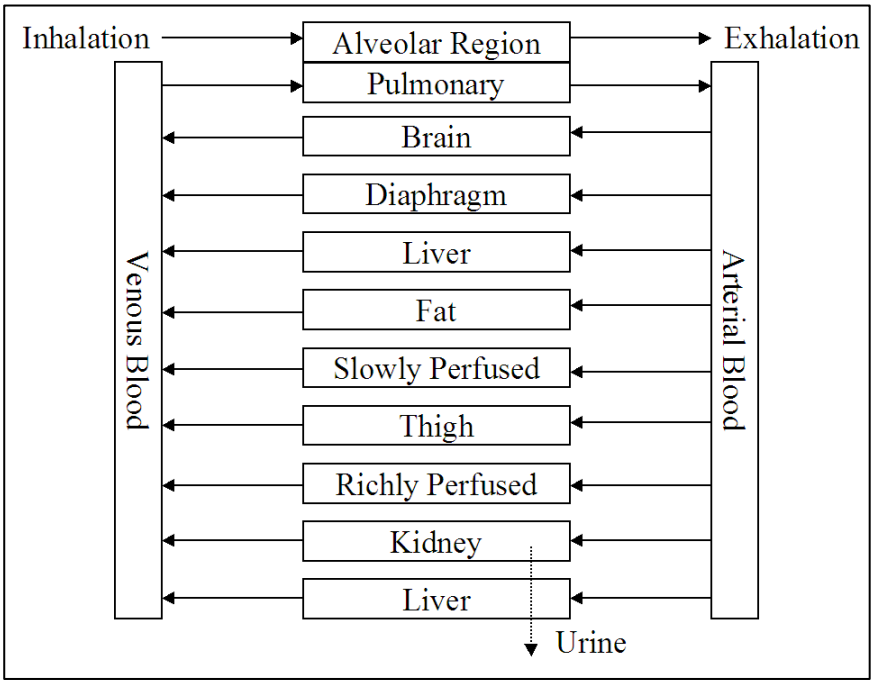

Figure 7. Model Structure

The model described organophosphate absorption by inhalation and described atropine and oxime absorption by bolus intramuscular injections in the thigh tissue. The model described chemical distribution by blood flow and described chemical excretion by loss with urine in the kidney compartment. Natural synthesis and degradation of esterases and acetylcholine molecules occurred solely within the compartments.

Chemical reactions among organophosphates, esterases, acetylcholine molecules, atropine, and oximes occurred within each compartment. However, esterase concentrations were assumed to be negligible or non-existent in fat tissue, and therefore, no chemical reactions occurred in the fat compartment.

Metabolism of organophosphates occurred by unspecified enzymes in all compartments except the fat compartment. In addition, degradation of organophosphates and esterases occurred by maturation of organophosphate-esterase bonds.

Equations

A complete list of equations is provided in Appendix A. All equations for absorption, distribution, metabolism, and excretion were primarily based on concepts used by Gearhart and others. All equations for chemical reactions were based on concepts presented by Clark.

Equation (3) describes the general inflow and outflow of organophosphates, atropine, and oximes for each compartment.

$$\begin{aligned} \textit{Accumulation} = {} & \textit{Inflow} + \textit{Intramuscular Injection} - \textit{Outflow} \\ & - \textit{Metabolism} - \textit{Urinary Excretion} \end{aligned} \qquad (3)$$

Natural synthesis and degradation of esterases occurred solely within the compartments and were described according to equation (4). Esterase levels were increased by a zero-order synthesis and decreased by first-order degradation.

$$\textit{Esterase Level} = \textit{Synthesis Constant} - \textit{Degradation Constant} * \textit{Enzyme Concentration} \quad (4)$$

Chemical reactions between organophosphates and esterases were modeled according to the following chemical reaction.

$$\text{Esterase} + \text{OP} \underset{l_2}{\overset{l_1}{<->}} \text{Esterase-OP Complex} \xrightarrow{l_3} \text{Aged Esterase-OP}$$

The chemical reaction was mathematically expressed according to equation (5).

$$d[\text{Esterase-OP}]/dt = k_1[\text{Esterase}][\text{OP}] - k_2[\text{Esterase-OP}] - k_3[\text{Esterase-OP}] \quad (5)$$

Chemical reactions between organophosphate-esterase complexes and oximes were based on the following chemical reaction.

$$\text{Esterase-OP} + \text{Oxime} \xrightarrow{o_1} \text{Esterase} + \text{OP} + \text{Oxime}$$

The chemical reaction was mathematically expressed according to equation (6).

$$-d[\text{Esterase-OP}]/dt = o_1[\text{Esterase-OP}][\text{Oxime}] \quad (6)$$

The model described a homeostatic condition of acetylcholine molecules at the binding sites with acetylcholine esterases. This condition represented one mole of acetylcholine molecules for every mole of acetylcholine esterases in each tissue compartment, except the fat compartment. The homeostatic condition was represented by a zero-order binding rate of acetylcholine molecules to acetylcholine esterases and a second-order hydrolysis of acetylcholine molecules by acetylcholine esterases.

$$d[ACh\text{-}AChE]/dt = p_1 - p_2[AChE][ACh\text{-}AChE] \quad (7)$$

Atropine does not directly interact with organophosphates or biological chemicals. Instead it reacts only with the neural receptor sites and limits acetylcholine's access to these sites. A simple ratio was incorporated into equation (7) that dampens the inflow of acetylcholine molecules to the binding sites and thereby integrates the effects of atropine.

$$d[ACh\text{-}AChE]/dt = p_1\{p_1/(p_1 + [Atropine])\} - p_2[AChE][ACh\text{-}AChE] \quad (8)$$

Equation (8) simplifies to equation (7) when no atropine is present. In this situation, the amount of acetylcholine molecules flowing into the binding sites is normal. If atropine is introduced, the net rate of acetylcholine molecules binding to acetylcholine esterases is reduced. If acetylcholine esterase levels drop as a result of chemical reactions with organophosphates, the outflow of acetylcholine molecules from equation (8) is dampened and results in a net increase of acetylcholine molecules at the binding sites.

Symptoms were described as a ratio between the amount of acetylcholine molecules at the acetylcholine binding sites and the basal level of acetylcholine molecules at the binding sites. This quantitative representation of symptoms was used as the primary index to compare therapeutic strategies.

$$dSymptoms/dt = [ACh\text{-}AChE\ site]/[Basal\ ACh\text{-}AChE\ site] \quad (9)$$

Assumptions

The model structure and the physiological mechanisms it is based on are drawn from current understanding of physiology, biological chemicals, organophosphates, and antidotes. Many of these mechanisms are vaguely or incompletely understood. In addition, the model attempts to simplify mechanisms where possible. Furthermore, much

pharmacokinetic data is incomplete for these chemicals. Although there are several minor assumptions, critical model assumptions are provided as follows.

1. Lumped-parameter distribution assumes instantaneous equilibration between tissue and blood and well-mixed distribution of the chemical within the interstitial fluid.
2. Metabolism of chemicals by the cytochrome P-450 enzyme suite follows Michaelis-Menten kinetics.
3. The release of acetylcholine from the pre-synaptic nerve cell and diffusion of the neurotransmitters across the synaptic cleft occurs so rapidly that it is assumed to be constant and continuous.

Parameters and Coefficients

Parameters and coefficients were either obtained from literature or fitted to the model to produce results that mimicked observations presented in literature. All parameters and coefficients used in the model are listed in Appendix B. Diisopropylfluorophosphate (DFP) data were obtained from Gearhart and others. This data were applied to the model to describe a typical organophosphate.

Very little pharmacokinetic data are available for atropine and oximes. Application of partition coefficients and metabolic constants from DFP data to describe the antidotes produced model results that generally mimicked limited observations of the antidotes described in research literature. In addition, a kidney elimination constant of 0.35 for the antidotes produced reasonable elimination of the antidotes, in whole, from the model with urine excretion.

Synthesis rates and initial amounts of esterases were obtained from Gentry and others. Degradation constants were calculated to maintain steady-state levels in each tissue compartment.

Acetylcholine molecules were assumed to continuously occupy all binding sites of acetylcholine esterases at a one-to-one molar ratio. Chemical reaction rate coefficients were calculated to maintain this ratio under normal conditions.

Bartling and others provided some reaction rate coefficients between esterases and organophosphates. It is known that butyrylcholinesterases have a higher affinity for organophosphates than acetylcholine esterases, and acetylcholine esterases have a higher affinity for organophosphates than carboxylesterases (Ashani and Pistinner 2004:365). The organophosphate reaction rate with butyrylcholinesterases and carboxylesterases was linked to the reaction rate with acetylcholine esterases. Butyrylcholinesterases were set to be twice as reactive with organophosphates as compared to acetylcholine esterases. Carboxylesterases were set to be half as reactive with organophosphates as compared to acetylcholine esterases.

Ashani and Pistinner suggested that acetylcholine esterase levels at 65% of basal levels were required to observe noticeable organophosphate induced symptoms, and acetylcholine esterase levels at 10% of basal levels were critical to life sustaining functions (Ashani and Pistinner 2004:365). In the model, an inhibition of acetylcholine esterases to 65% of basal levels produced a symptom level of 1.09, and an inhibition of acetylcholine esterases to 10% of basal levels produced a symptom level of 1.16. With no inhibition of acetylcholine esterases, the standard symptom level was 1.0.

Literature suggested that atropine doses of 10mg without organophosphate poisoning also produced adverse symptoms (USAMRICD 2000:120). Application of 10 mg of atropine to the model produced a peak atropine level of 0.08 mg in the brain compartment at 9 minutes and corresponded to a symptom level of 0.89. The symptom level continued to decline to a low value of 0.45 at 97.8 minutes. It was assumed that symptom levels below 0.91 produced adverse effects from atropine and symptom levels below 0.84 for 10 minutes or longer produced death from atropine.

Sensitivity Testing

For sensitivity testing, 10 mg of organophosphate per liter of air was applied to the model, beginning at time 0, for 15 minutes. In addition, 2 mg of atropine and 600 mg of oxime were added at time 0. The maximum value of the symptom level was measured and recorded. Each of the model parameters and coefficients was changed to a 50% and 150% value. The model was individually run for each change in parameters and coefficients, and the maximum value of the symptom level was recorded.

The greatest variability of symptoms rested with the reaction rate coefficient between acetylcholine esterases and the organophosphate. This result would show strong support that variability of organophosphate toxicity among different types of organophosphates rests greatest with the reaction rates between the organophosphates and acetylcholine esterases. Of less variability, but noticeable, were the partition coefficients of the organophosphate. As the partition coefficients of the organophosphate increased, organophosphate persisted longer in the tissue compartments and reacted with more acetylcholine esterases. In addition, the partition coefficients of atropine also had a noticeable impact on symptom levels. As the partition coefficients of atropine increased,

atropine persisted in the tissue compartments and countered the effects of excessive neural stimulation for a longer period of time.

Although the partition coefficients of the organophosphate had an impact on the symptom level, the DFP data provided by Gearhart and others was used as the standard organophosphate, representing all varieties of organophosphorus chemicals. Consequently, the major factors that would affect the differences in toxicity of organophosphates in the model would result from the different reaction rate coefficients with acetylcholine esterases.

Although the partition coefficients of atropine affected symptom levels, the applied parameter values reasonably mirrored anecdotal observations of atropine. Furthermore, there is no variability among a class of atropine as there is with organophosphates. Therefore, maintaining atropine parameters at the values initially suggested is reasonable and permits fair comparison among the tests.

Test Protocol

Initially, two types of theoretical organophosphates were created for the study. The first organophosphate reacted rapidly with acetylcholine esterases, and the organophosphate-acetylcholine esterase bonds matured quickly and responded poorly to oximes. In comparison, the second organophosphate reacted slowly with acetylcholine esterases, and organophosphate-acetylcholine esterase bonds matured slowly and responded favorably to oximes.

A series of 19 tests were run for each organophosphate type. The tests are described in Table 3 of Appendix C. Series A tests incorporated the more toxic organophosphate and Series B tests incorporated the less toxic organophosphate. For each

test, the time symptoms first appeared, the length of time symptoms persisted, and the total amounts of antidotes administered were recorded. Each test applied an organophosphate exposure of 5 mg per liter of air for 15 minutes beginning at +5 minutes.

IV. Results and Analysis

Appendix C provides all test protocols and detailed results.

Series A and B Tests

The organophosphate exposure depressed acetylcholine levels and increased symptom levels for both series of tests. Noticeable symptoms appeared at approximately 20 minutes for Series A tests and at 35 minutes for Series B tests after organophosphate exposure began.

Increasing and decreasing rates of symptom levels at the end of 3 hours were dependent on the amount of atropine in the tissue at that time. Therefore, it is expected that all symptom levels will eventually return to higher levels beyond 3 hours until acetylcholine esterase levels return to normal.

Atropine appeared to have immediate impacts on suppressing symptom levels. In tests, where only atropine was used, symptom levels remained above noticeable levels for only 1.2 to 8.4 minutes after the first injection of atropine.

In general, strictly time-based dosing strategies for atropine appear excessive and wasteful. In both series, where only atropine was used, continuous interval dosing of atropine led to a build-up of the antidote in the tissue and a prolonged level of critical symptoms resulting from atropine toxicity. Conversely, it would seem that longer dosing intervals between atropine administrations would risk the scenario of not administering enough atropine to suppress organophosphate induced symptoms. In addition, after

comparing the tests between the two series, the duration of symptom levels with the presence of atropine varied with the organophosphate toxicity.

In just three hours, 56 to 72 mg of atropine were needed in a time-based dosing strategy, which corresponds to 28 to 36 injections of atropine. A symptoms-based dosing strategy for the same organophosphate exposures, required only 2 to 4 mg of atropine, corresponding to 1 to 2 injections of atropine, to keep symptoms suppressed. The symptoms-based dosing strategy required 1% to 3% of the total atropine used in the time-based dosing strategy and did not produce atropine toxicity.

Administering atropine before the onset of organophosphate exposure appears to delay the onset of symptoms. In Series A, a pre-dose administration of atropine delayed onset of symptoms by 69.2 minutes as compared to administering atropine upon the appearance of noticeable symptoms. Similar results were found when comparing tests of Series B.

Oxime treatment yielded poor results. In tests where only oximes were administered, the oximes failed to suppress symptom levels. It is possible that the organophosphates used in the tests were too toxic for the oximes to overcome.

There is a delay between the time organophosphate exposure begins and the first observation of symptoms. Symptoms appeared nearly 20 minutes after the onset of organophosphate exposure. This delay between exposure and symptoms results from the fact that acetylcholine esterase levels must be degraded to 65% of basal levels before symptoms first appear. The model was constructed to ensure oximes break organophosphate-acetylcholine esterase bonds. However, oximes are at a severe time disadvantage in performing their function when they are introduced at the onset of

symptoms. A full 20 minutes would elapse between organophosphate exposure and symptoms induced oxime injection. As a result, some organophosphate-acetylcholine esterase bonds can mature before oximes ever have a chance to perform their function.

Atropine is at a similar disadvantage, but to a less severe degree. First atropine's therapeutic effect is not directly dependent on acetylcholine esterase levels. Second, as noted earlier, atropine reacts quickly to suppress symptoms.

Another possibility for the failure of oximes may rest with the understood mechanisms of organophosphate toxicity, and therefore, the structure of the model. The model was created to allow organophosphates the ability to rebind with acetylcholine esterases after the organophosphates were freed by oximes from initial bonds with acetylcholine esterases. In this model structure, a mass of organophosphate and a mass of acetylcholine esterase will bind together during a single time increment. For the next time increment, some of the organophosphate-acetylcholine esterase mass ages and some of the organophosphate-acetylcholine esterase mass is broken apart by the oxime. For the third time increment, some of the freed acetylcholine esterases and organophosphates immediately re-bind. This cycle repeats, and the oxime is in a losing battle since a portion of the organophosphate-acetylcholine esterase mass ages with each time increment.

Series C Tests

To explore the possibility that a continuous cycle of binding and rebinding between organophosphates and acetylcholine esterases mitigates or negates the effects of oximes, the model was restructured so that freed organophosphates by oximes were unable to rebind with acetylcholine esterases. Under this new model structure, the tests in

Series B were repeated. The results for Series C tests were identical to the results of Series B tests. Again, oximes were unable to suppress symptom levels.

Series D and E Tests

The inability of oximes to reduce symptom levels or the onset of symptoms was disturbing. As a result, additional tests were undertaken to determine if the organophosphate toxicity was too severe in comparison to the oxime therapeutic ability.

The new test protocols varied the reaction rate coefficients among the chemicals and applied an organophosphate exposure and a time-based dosing strategy for oxime treatment. Series D tests used the model structure in which freed organophosphates were unable to reactivate with acetylcholine esterases once the organophosphates were freed from bonds with the esterases by oximes. Series E tests used the model structure in which freed organophosphates were able to reactivate with acetylcholine esterases once the organophosphates were freed from bonds with the esterases by oximes.

Oximes show some efficacy in both test series when the aging rate of the organophosphate-acetylcholine esterase bonds was significantly lowered. For Series D, oximes reduced the destruction of acetylcholine esterases by nearly 11.5% as compared to the same organophosphate exposure with no oxime dose. For series E, oximes reduced the destruction of acetylcholine esterases by approximately 9.8% as compared to the same organophosphate exposure with no oxime dose. These results suggest two findings. First, oximes are slightly more effective if the organophosphates are unable to rebind with acetylcholine esterases after the organophosphates are freed from bonds with the esterases by oximes. Second, oxime efficacy is sensitive to the organophosphate-acetylcholine esterase maturation rate coefficient.

Series F Tests

For Series F tests, more attention was given to the maturation rate coefficients. The maturation half-lives of some nerve agents are well known. Soman-acetylcholine esterase bonds have an maturation half-life of less than 10 minutes, sarin-acetylcholine esterase bonds have an maturation half-life of 5 hrs, and VX-acetylcholine esterase bonds have an maturation half-life of approximately 48 hours (Cannard 2006:89).

Using equation (10), λ was calculated for half-lives of 10 minutes, 5 hours, and 48 hours. With equation (11), the maturation rate coefficients were then found through model fitting.

$$Half\text{-}Life = .693/\lambda \tag{10}$$

$$A = A_o e^{-\lambda t} \tag{11}$$

Furthermore, the reaction rate coefficients between organophosphates and acetylcholine esterases were refined based on literature data. Thiermann and others present reaction rate coefficients of 132,000 $mmol^{-1}hr^{-1}$ and 1320 $mmol^{-1}hr^{-1}$ for two organophosphorus insecticides (Thiermann and others, 1999:234). Bartling and others provide reaction rate coefficients for some nerve agents ranging between 660,000 $mmol^{-1}hr^{-1}$ and 31,800,000 $mmol^{-1}hr^{-1}$ (Bartling and others, 2007:169). Using the calculated maturation rate coefficients and using the reaction rate coefficients between organophosphates and acetylcholine esterases of 1,000,000 $mmol^{-1}hr^{-1}$ and 50,000 $mmol^{-1}hr^{-1}$, organophosphates and oximes were applied to the model.

There were four significant findings from this series of tests. First, when the amount of organophosphates and time of organophosphate exposure is increased, the efficacy of oximes is reduced. Second, when oximes are administered before organophosphate exposure begins, more acetylcholine esterases are recovered over time. Third, oximes appear to have no positive effect on suppressing symptoms when the reaction rate between organophosphates and acetylcholine esterases is relatively high and the maturation half-life of organophosphate-acetylcholine esterase bonds is also high. Finally, in certain conditions, oximes appear to make acetylcholine esterase levels lower and symptom levels higher than if no oximes were administered at all.

The last finding is illustrated in Figure 8. Figure 8 depicts the test in which 5 mg / L of organophosphate was applied for 15 minutes from time +5 to time +20 minutes. In this test, the reaction rate coefficient between organophosphates and acetylcholine esterases was relatively high and the maturation half-life of organophosphate-acetylcholine esterase bonds was 5 hours. An oxime injection of 600 mg was administered upon the first appearance of noticeable symptoms and 600 mg of oxime were administered every 5 minutes thereafter. In this graph, acetylcholine esterase levels immediately dropped with the presence of organophosphate exposure. With the administration of oximes, acetylcholine esterase levels dropped further and then began to improve.

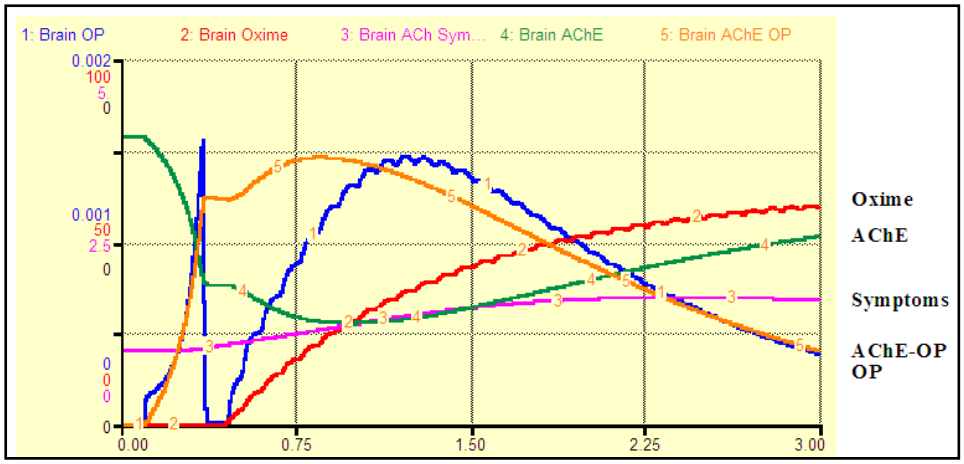

Figure 8. Organophosphate Exposure with Continuous Oxime Treatment

When only one injection of 600 mg of oximes was administered at the appearance of noticeable symptoms, acetylcholine esterase levels dropped and failed to improve. This scenario is illustrated in Figure 9 and suggests that oximes made organophosphate poisoning worse.

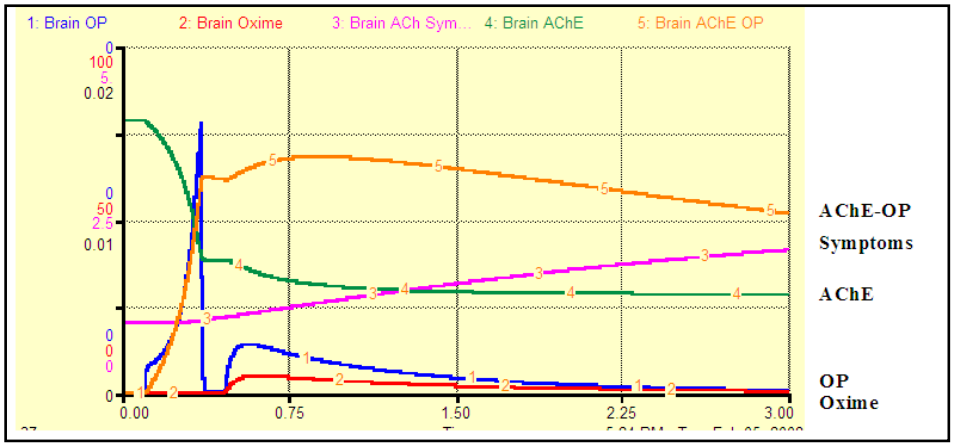

Figure 9. Organophosphate Exposure with One Oxime Injection

One of the reasons why the oximes may make acetylcholine esterase levels worse is the fact that the reaction rate coefficients between oximes and esterases vary. The organophosphate's affinity for esterases decreases, in order, with butyrylcholinesterases, acetylcholine esterases, and carboxylesterases. There is a potential that the oxime is

breaking organophosphate-esterase bonds, and that a higher proportion of organophosphates, originally bound to carboxylesterases, are now binding with butyrylcholinesterases and acetylcholine esterases. If this mechanism is true, carboxylesterase levels should increase with time after the injection of oximes. Figure 10 shows the esterase levels for the same organophosphate exposure and one injection of 600 mg of oxime. All three esterase levels decrease to lower levels after the injection of oxime. Therefore, although organophosphates released from bonds with carboxylesterases may be disproportionately rebinding with acetylcholine esterases and butyrylcholinesterases, it is not the determining cause of lower acetylcholine esterase levels.

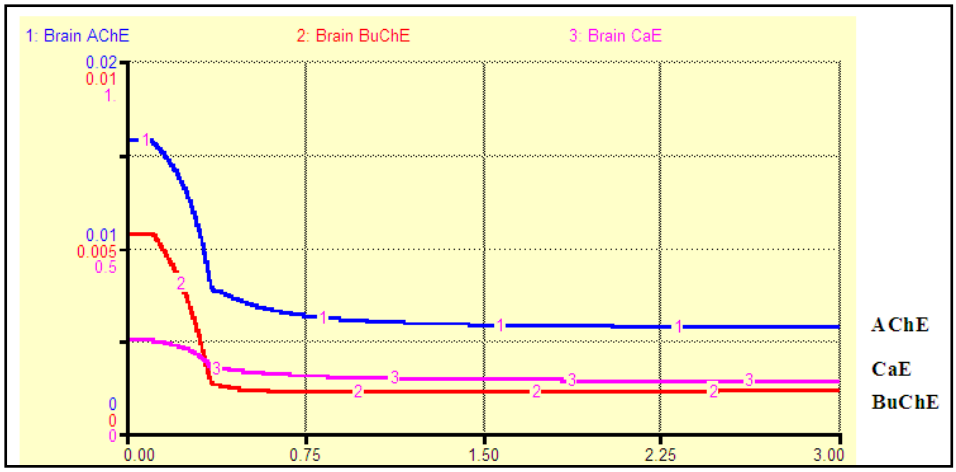

Figure 10. Esterase Levels in Brain Compartment

Figure 11 shows carboxylesterase levels in other tissue compartments of the model. As with Figure 10, carboxylesterase levels are decreasing after the injection of oximes.

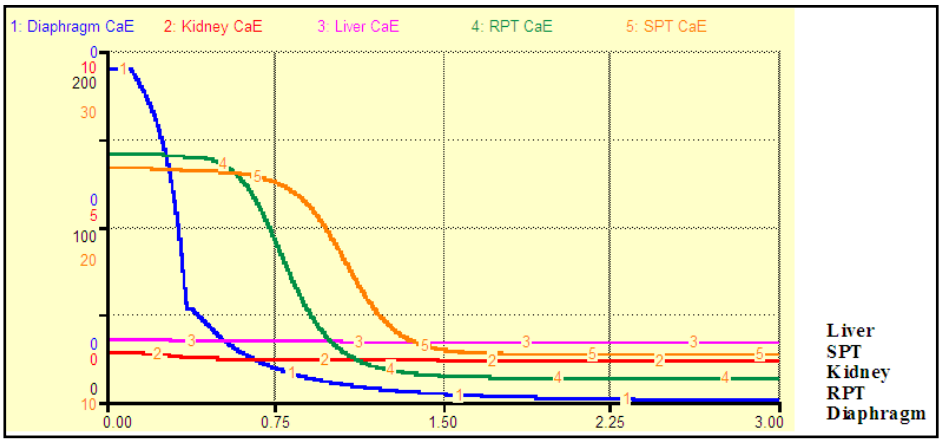

Figure 11. Carboxylesterase Levels in Various Tissue Compartments

Figure 12 shows esterase levels in the arterial compartment. The figure shows that all esterase levels improve after the oxime injection. Therefore, it appears that oximes are effectively breaking organophosphate-enzyme bonds in the arterial compartment and restoring some levels of these esterases. However, the released organophosphate is diffusing into the other tissue compartments and lowering esterase levels in those compartments.

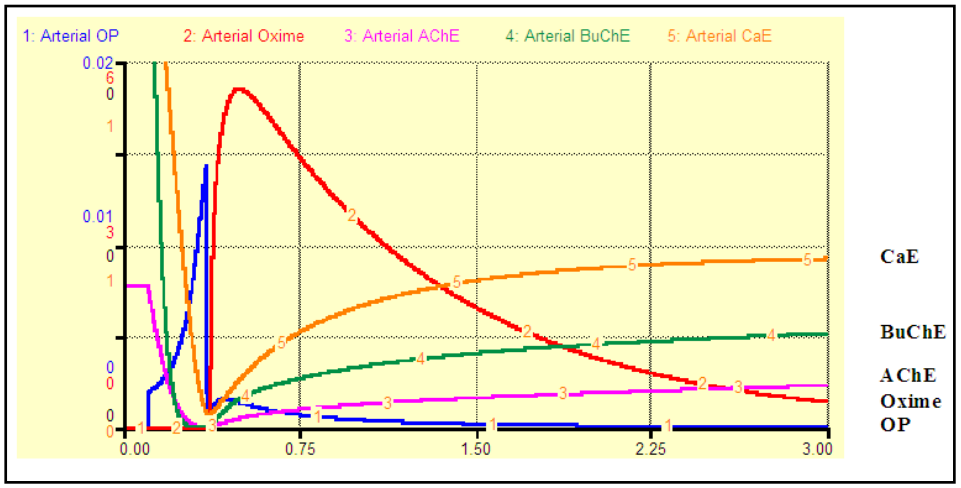

Figure 12. Esterase Levels in Arterial Compartment

The phenomenon that oximes may make organophosphates poisoning worse results from physiology and the model structure. Organophosphates enter the model through the lung compartment, proceed through the arterial compartment, and then pass through tissue compartments. Esterases in the arterial compartment are the first to react with and most severely affected by organophosphates.

This reasoning was explored by Ashani and Pistinner in 2003. In their study, the researchers used exogenous butyrylcholinesterases as a prophylactic against organophosphate poisoning. The researchers believed that since butyrylcholinesterases have a higher affinity to organophosphates, excess butyrylcholinesterase levels in the blood would react in greater numbers with organophosphates. As a result, fewer organophosphates would react with acetylcholine esterases in the blood and fewer organophosphates were able to diffuse into the tissue compartments. (Ashani and Pistinner, 2003:358-367)

This same reasoning may explain why oximes are causing acetylcholine esterase levels to drop to lower levels in the tissue compartment. Organophosphate-esterase bonds in the blood are the first benefited by oximes. The oximes are improving esterase levels in the blood, but freed organophosphates are diffusing into tissue compartments and reacting with esterases in these tissue compartments.

Research literature supports this finding. In a 2007 article, Szinicz and others reviewed the efficacy of oxime treatment. The authors stated “human reports of severe toxicity frequently mention failure of oxime therapy and conclude oximes to be ineffective, or even harmful” (Szinicz and others, 2007:25). Furthermore, citing other

studies, the authors note that “disappointing results were found when … oxime administration was discontinued prematurely” (Szinicz and others, 2007:27).

Szinicz and other suggested that the failure of oximes may result from freed esterases rebinding with persisting organophosphates in the body (Szinicz and others, 2007:26). This suggested mechanism may complement the causes of oxime failure. However, the model suggests that there are three determining and interdependent causes of oxime failure for highly toxic organophosphates. First, the antidote disproportionately breaks more organophosphate-esterase bonds in the blood as compared to organophosphate-esterase bonds the tissue groups. Second, the freed organophosphates in the blood are rebinding in greater numbers with esterases in the tissue groups. Finally, the oxime reaction rate to break organophosphate-esterase bonds and the time elimination of oximes from the body cannot overcome the affinity of organophosphates for esterases.

Although laboratory procedures to measure organophosphates poisoning and acetylcholine esterase levels were not included in the scope of this study, the model suggested interesting findings that may be relevant for these procedures. Scientists and medical doctors typically measure acetylcholine esterase levels in the blood to determine the severity of organophosphate poisoning and the efficacy of oximes. The model suggests that this measurement technique may provide misleading results. The oxime may be improving esterase levels in the blood while simultaneously lowering esterase levels in tissue groups, where acetylcholine esterases are more critical to proper physiological functioning.

V. Discussion

Research Objectives

This research set out to accomplish four objectives.

1. Construct a physiologically based pharmacokinetic model (PBPK) to predict the tissue concentrations of organophosphates, atropine, oximes, and pertinent biological chemicals.

Although very little data are available to accurately predict tissue concentrations for atropine and oxime, model fitting the parameters reasonably represented literature observations. In addition, the partition coefficients and metabolic constants were available for only a few organophosphates. A sensitivity test revealed that the parameters for all of these chemicals had little impact on the outcome of human response. Research revealing more accurate constants would likely produce more refined results. Such refinement, however, given the results of the sensitivity analysis, would not be expected to alter the conclusions of this work.

The PBPK model could incorporate two additional elements of organophosphate poisoning that may provide beneficial information. First, the entry routes of organophosphates could include ingestion and dermal absorption. Second, the toxic effects of some organophosphates, such as parathion, result from the metabolite of the chemical and not the chemical itself. Modeling these intake routes and metabolites would likely alter time distribution of the chemical and the severity and onset of symptoms.

2. Integrate the reactions among these chemicals into the model and produce a quantitative measurement of their aggregate effects.

The equations to represent accumulation of acetylcholine molecules at the neural synapse and symptom levels appeared to produce results that followed observed outcomes of organophosphate poisoning. The equations were simple and allowed for easy comparison among different organophosphate exposures and therapeutic strategies. However, it is important to note that atropine and oximes mitigate different symptoms of organophosphate poisoning, because the antidotes work at different types of neural receptors. The tests conducted in this work included administering atropine and oximes separately; and therefore, future refinement of the equations would not be expected to alter the conclusions of this work.

The reactions among the chemicals appear to have had the most dominant affect on the outcome of acetylcholine esterase and symptom levels. Applying the entire variety of kinetic reaction rate constants will likely help to refine the efficacy of oximes in relation to the various organophosphates. However, as with the chemical parameters, such refinement would not be expected to alter the major conclusions of this work.

3. Apply therapeutic strategies for atropine and oximes against an organophosphate exposure to the model.

This thesis reviewed time-based dosing of antidotes, symptoms-based dosing of antidotes, administering antidotes upon the appearance of symptoms, and administering antidotes before the onset of organophosphate exposure. Symptoms-based dosing of atropine proved most economical and ensured correct amounts of the antidote were administered to properly treat organophosphate poisoning and prevent adverse effects of

atropine toxicity. Although agencies may find it easier to mass educate a time-based dosing strategy for atropine, allocating resources to educate medical technicians and military personnel about the intricacies of organophosphate induced symptoms may better serve the patient and conserve the medication.

In certain scenarios, when oximes were effective, a time-based dosing strategy of oximes proved most prudent. There is a delay between the time organophosphates bind to acetylcholine esterases and the appearance of symptoms. During this time delay, some organophosphate-acetylcholine esterase bonds begin to mature. It is imperative that oximes are introduced early and maintain presence in the tissue groups until organophosphates are completely removed from the body. A time-based application of oximes would maintain oxime levels between an ideal range in the tissue groups. Personnel at medical facilities could ensure the ideal range of oxime levels in the tissue groups persisted until the removal of all organophosphates from the body.

Current procedures for medical facilities to administer antidotes are slightly different than procedures for first care responders. Medical facilities have the ability to administer the antidotes intravenously, normally have greater quantities of the antidotes, and will potentially have to manage a patient for weeks until the full recovery of acetylcholine esterases. It is possible the dynamics of organophosphate poisoning and the optimal strategies for antidote administration are slightly different for long-term treatment than what has been suggested in this study. Investigation into this long-term treatment may be worthy for future research.

4. Compare model output and report significant differences among therapeutic strategies.

Test results revealed five important findings. First, application of antidotes before organophosphate exposure began tended to delay the onset and reduce the severity of symptom levels. Although this finding is beneficial, the ability of individuals to anticipate organophosphate exposure and preempt the poisoning with antidote treatment is highly improbable.

Second, oxime efficacy decreases with the exposure duration and toxicity of organophosphates. Although this may be an obvious and expected finding, the finding does suggest that when the severity of organophosphate poisoning reaches a certain level, oximes may no longer be effective in recovering any significant amounts of acetylcholine esterases. This point was shown when an organophosphate with a kinetic reaction rate constant similar to nerve agents and an aging half-life of 10 minutes was applied to the model. Excessive oxime doses failed to recover any noticeable amounts of acetylcholine esterases.

Third, oximes efficacy improves if organophosphates cannot reactivate with acetylcholine esterases once the organophosphates are freed by oximes from the esterases. The ability for organophosphates to rebind with acetylcholine esterases is suspected but is not definitively known for all organophosphate types.

Fourth, there is a time delay between organophosphate exposure and the appearance of noticeable symptoms. The time delay varies with the toxicity of the organophosphate and the duration of organophosphate exposure. The time delay appears to have no significant bearing on atropine efficacy. The benefits of atropine appear largely independent of acetylcholine esterase levels and occur rather quickly. If oximes are introduced when symptoms first appear, there is a potential that irreversible

maturation of the organophosphate-acetylcholine esterase bond has already occurred. For the condition when the aging half-life of organophosphate-acetylcholine esterase bonds is relatively high, it is unlikely that copious doses of oximes will have any positive effect on recovery of acetylcholine esterase and reducing symptom levels. However, oximes could be significantly effective if oximes are introduced when symptoms first appear and if the organophosphate-acetylcholine esterase aging rate coefficient is relatively low. Under this condition, it is likely that oximes will help restore some, and possibly significant, portions of acetylcholine esterase to a functional state.

Finally, under certain conditions, such as when the kinetic reaction rate coefficient between organophosphates and acetylcholine esterases is relatively high and the aging half-life of organophosphate-acetylcholine esterase bonds is moderate, oximes depress acetylcholine esterase levels in tissue groups. The model suggests that oximes effectively free organophosphate-enzyme bonds in the arterial compartment. However, freed organophosphates diffuse into the other tissue groups, quickly react with esterases, and cause esterase levels to decrease in those tissue groups. If continuous doses of oximes are applied, acetylcholine esterase levels will eventually improve. However, enough oxime must be continuously administered and the body must be able to tolerate the large quantities of required oximes to achieve a positive effect.

Recommendations

There are two likely situations that involve organophosphate poisoning. First, people living and working in rural, agricultural areas may become poisoned through improper application and accidental exposure to organophosphorus insecticides. These organophosphates tend to have low reaction rate coefficients with acetylcholine esterases

and moderate to low aging half-lives of organophosphate-acetylcholine esterase bonds. The model suggests that first care responders should administer atropine on a symptoms-based dosing strategy to ensure proper suppression of symptoms and limit adverse affects of atropine toxicity. In addition, first care responders should administer oximes early and continuously until further tests and time assure the complete removal of organophosphates from the body. Oximes appear quite effective for relatively weak organophosphates.

Organophosphate poisoning for people living in urban areas and military personnel involved in conflicts will likely result from either a terrorist event or a military weapon. It is highly probably that these organophosphates are of the nerve agent variety, which have a high reaction rate coefficient with acetylcholine esterases. Again, the model suggests that symptoms-based dosing of atropine is the most economical use of the antidote. In addition to properly treating the patient, conservation of atropine could be crucial when atropine supplies are limited. A terrorist event could involve mass casualties and deployed military personnel only carry 6 mg of atropine on their persons. Furthermore, the model suggests first care responders to a terrorist event or military personnel in conflict should not administer oximes. It is likely that the oximes are not effective, and in some cases, cause the severity of organophosphate toxicity to increase.

Appendix A. Equations

Organophosphates

Slowly Perfused, Thigh Tissue, Diaphragm Tissue, and Fat Tissue

$dA/dt = F * CA - F * QC * CV / BW / NF / P$

Brain, Liver, Kidney, and Rapidly Perfused Tissue

$dA/dt = F * CA - F * QC * CV / BW / NF / P - (Vmax * CV / BW / NF)/(KM + CV / BW / NF)$

Venous Compartment

$dA/dt = E (CV) - QC * CV_1 / P - (Vmax * CV_1 / BW / NF)/(KM + CV_1 / BW / NF)$

Lung Compartment

$QP * Cexp + CV_1 = QP * CA1 / P + QC * CA_1$

Arterial Compartment

$dA/dt = QC \times CA_1 - QC \times CA / P - (Vmax * CV / BW / NF)/(KM + CV / BW / NF)$

Oxime

Brain, Diaphragm, Fat, Richly Perfused, Slowly Perfused, and Thigh Tissues

$dA/dt = F * CA - F * QC * CV / BW / NF / P$

Kidney Tissue

$dA/dt = F * CA - F * QC * CV / BW / NF / P - EP * CV$

Liver Tissue

$dA/dt = F * CA - F * QC * CV / BW / NF / P - (Vmax * CV / BW / NF)/(KM + CV / BW / NF)$

Venous Compartment

$dA/dt = E (CV) - QC * CV/ P$

Lung Compartment

$QP * Cexp + CV_1 = QP * CA1 / P + QC * CA_1$

Arterial Compartment

$dA/dt = QC \times CA_1 - QC \times CA / P$

Atropine

Brain, Diaphragm, Fat, Richly Perfused, Slowly Perfused, and Thigh Tissues

$dA/dt = F * CA - F * QC * CV / BW / NF / P$

Kidney Tissue

$dA/dt = F * CA - F * QC * CV / BW / NF / P - EP * CV$

Liver Tissue

$dA/dt = F * CA - F * QC * CV / BW / NF / P - (Vmax * CV / BW / NF)/(KM + CV / BW / NF)$

Venous Compartment

$dA/dt = E(CV) - QC * CV/ P$

Lung Compartment

$QP * Cexp + CV_1 = QP * CA_1 / P + QC * CA_1$

Arterial Compartment

$dA/dt = QC \times CA_1 - QC \times CA / P$

Acetylcholinesterase

Brain, Kidney, Diaphragm, Liver, Slowly Perfused, Richly Perfused, and Thigh Tissues

$dA/dt = X_1 - X_2A$

Butyrylcholinesterase

Brain, Kidney, Diaphragm, Liver, Slowly Perfused, Richly Perfused, and Thigh Tissues

$dA/dt = Y_1 - Y_2A$

Carboxylesterase

Brain, Kidney, Diaphragm, Liver, Slowly Perfused, Richly Perfused, and Thigh Tissues

$dA/dt = Z_1 - Z_2A$

Acetylcholinesterase and Organophosphate Chemical Reaction

$$\text{AChE} + \text{OP} \underset{L_2}{\overset{L_1}{<\!\!-\!\!-\!\!-\!\!>}} \text{AChE/OP} \xrightarrow{L_3} \text{Aged AChE/OP}$$

$d[\text{AChE/OP}]/dt = L1 * [\text{AChE}] * [\text{OP}] - L_2 [\text{AChE/OP}] - L_3 [\text{AChE/OP}]$

Butyrylcholinesterase and Organophosphate Chemical Reaction

$$\text{BuChE} + \text{OP} \underset{M_2}{\overset{M_1}{<\!\!-\!\!-\!\!-\!\!>}} \text{BuChE/OP} \xrightarrow{M_3} \text{Aged BuChE/OP}$$

$d[\text{BuChE}]/dt = M_1 * [\text{BuChE}] * [\text{OP}] - M_2 * [\text{BuChE/OP}] - M_3 * [\text{BuChE/OP}]$

Carboxylesterase and Organophosphate Chemical Reaction

$$\text{CaE} + \text{OP} \underset{N_2}{\overset{N_1}{<\!\!-\!\!-\!\!-\!\!>}} \text{CaE/OP} \xrightarrow{N_3} \text{Aged CaE/OP}$$

$d[\text{CaE/OP}]/dt = N_1 * [\text{CaE}] * [\text{OP}] - N_2 [\text{CaE/OP}] - N_3 [\text{CaE/OP}]$

Oxime and AchE/OP Chemical Reaction

$$\text{AChE/OP} + \text{Oxime} \xrightarrow{O_1} \text{AChE} + \text{OP} + \text{Oxime}$$

$-d[\text{AChE/OP}]/dt = O_1 * [\text{AChE/OP}] * [\text{Oxime}]$

Oxime and BuChE/OP Chemical Reaction

$$\text{BuChE/OP} + \text{Oxime} \xrightarrow{P_1} \text{BuChE} + \text{OP} + \text{Oxime}$$

$$-d[\text{BuChE/OP}]/dt = P_1 * [\text{BuChE/OP}] * [\text{Oxime}]$$

Oxime and CaE/OP Chemical Reaction

$$\text{CaE/OP} + \text{Oxime} \xrightarrow{R_1} \text{CaE} + \text{OP} + \text{Oxime}$$

$$-d[\text{CaE/OP}]/dt = R_1 * [\text{CaE/OP}] * [\text{Oxime}]$$

Atropine, Acetylcholine, and Acetylcholine Esterase Reaction

$$d[\text{ACh-AChE}]/dt = p_1\{p1/(p_1 + [\text{Atropine}])\} - p_2[\text{AChE}][\text{ACh-AChE}]$$

Symptoms

$$d\text{Symptoms}/dt = [\text{ACh-AChE site}]/[\text{Basal ACh-AChE site}]$$

Legend

dA/dt = change in chemical accumulation with respect to time (mass / time)
F = Fraction of blood flow that enters the tissue (unitless)
CA = Arterial Blood Outflow (mass / time)
QC = Cardiac Output (volume / time)
CV = Mass of OP in tissue (mass)
BW = Body Weight (mass)
NF = Normalization Factor (volume of tissue / mass of body weight)
P = Tissue to blood partition coefficient (unitless)
KM = Michaelis-Menten Constant (mass / volume)
Vmax = Maximum metabolism rate of OP (mass / time)
CA_1 = arterial concentration (mass / volume)
QP = pulmonary ventilation rate (volume / time)
Cexp = concentration of chemical in alveolar space (mass / volume)
CV_1 = venous blood output (mass / time)
P = air to blood partition coefficient (unitless)
QC = cardiac output (volume / time)
K = conversion factor to convert kg of air to L of air = 0.001204 kg of air / L of air
EP = Elimination parameter of pure oxime into urine. ($time^{-1}$)
X_1 = AChE synthesis rate (mass / time)
X_2 = AChE degradation constant ($time^{-1}$)
Y_1 = BuChE synthesis rate (mass / time)
Y_2 = BuChE degradation constant ($time^{-1}$)
Z_1 = CaE synthesis rate (mass / time)
Z_2 = degradation constant ($time^{-1}$)
p_1 = Acetylcholine binding rate (mass / time)
p_2 = Acetylcholine degradation constant ($time^{-1}$)
O_1 = Oxime reaction rate coefficient for AChE ($mass^{-1}time^{-1}$)
P_1 = Oxime reaction rate coefficient for BuChE ($mass^{-1}time^{-1}$)

R_1 = Oxime reaction rate coefficient for CaE ($mass^{-1}time^{-1}$)
L_1 = OP reaction rate coefficient with AChE ($mass^{-1}time^{-1}$)
L_2 = OP-AChE natural separation coefficient ($time^{-1}$)
L_3 = OP-AChE aging coefficient ($time^{-1}$)
M_1 = OP reaction rate coefficient with BuChE ($mass^{-1}time^{-1}$)
M_2 = OP-BuChE natural separation coefficient ($time^{-1}$)
M_3 = OP-BuChE aging coefficient ($time^{-1)}$
N_1 = OP reaction rate coefficient with CaE ($mass^{-1}time^{-1}$)
N_2 = OP-CaE natural separation coefficient ($time^{-1}$)
N_3 = OP-CaE aging coefficient ($time^{-1}$)

Appendix B. Parameters

Physiological Parameters		
Body Weight	60.9 kg	Gearhart et. al.
Cardiac Output	302 L/hr	Gearhart et. al.
Pulmonary Rate	354 L/hr	Gearhart et. al.
Blood Flow to Tissue Fractions		
Arterial	1	Assumed
Brain	0.134	Gearhart et. al.
Diaphragm	0.006	Gearhart et. al.
Richly Perfused	0.2	Gearhart et. al.
Fat	0.036	Gearhart et. al.
Slowly Perfused	0.1244	Gearhart et. al.
Thigh	0.0066	Gearhart et. al.
Kidney	0.223	Gearhart et. al.
Liver	0.27	Gearhart et. al.
Venous	1	Assumed
Tissue Normalization Factors		
Arterial	0.02 L/kg	Gearhart et. al.
Brain	0.0214 L/kg	Gearhart et. al.
Diaphragm	0.003 L/kg	Gearhart et. al.
Richly Perfused	0.0343 L/kg	Gearhart et. al.
Fat	0.17 L/kg	Gearhart et. al.
Slowly Perfused	0.5238 L/kg	Gearhart et. al.
Thigh	0.0276 L/kg	Gearhart et. al.
Kidney	0.0043 L/kg	Gearhart et. al.
Liver	0.04 L/kg	Gearhart et. al.
Venous	0.057 L/kg	Gearhart et. al.
Organophosphate		
Molecular Weight	184 mg/mmol	Calculated
Partition Coefficients		
Brain	0.67	Gearhart et. al.
Diaphragm	0.77	Gearhart et. al.
RPT	0.67	Gearhart et. al.
Fat	17.6	Gearhart et. al.
SPT	0.77	Gearhart et. al.
Thigh	0.77	Gearhart et. al.
Kidney	1.63	Gearhart et. al.
Liver	1.53	Gearhart et. al.
Arterial	1	Assumed

Venous	1	Assumed
Blood/Air	12.57	Gearhart et. al.
Metabolic Parameters by A Esterases		
Brain Vmax	688 mg/hr	Gearhart et. al.
Brain KM	440 mg/L	Gearhart et. al.
RPT Vmax	560 mg/hr	Gearhart et. al.
RPT KM	51 mg/L	Gearhart et. al.
Kidney Vmax	5042 mg/hr	Gearhart et. al.
Kidney KM	134 mg/L	Gearhart et. al.
Liver Vmax	52474 mg/hr	Gearhart et. al.
Liver KM	237 mg/L	Gearhart et. al.
Venous Vmax	616 mg/hr	Gearhart et. al.
Venous KM	199 mg/L	Gearhart et. al.
Arterial Vmax	216 mg/hr	Gearhart et. al.
Arterial KM	199 mg/L	Gearhart et. al.
Oxime		
Molecular Weight	132 mg/mmol	Heath and McKeown
Partition Coefficients		
Brain	0.67	Assumed
Diaphragm	0.77	Assumed
RPT	0.67	Assumed
Fat	17.6	Assumed
SPT	0.77	Assumed
Thigh	0.77	Assumed
Kidney	1.63	Assumed
Liver	1.53	Assumed
Venous	1	Assumed
Arterial	1	Assumed
Blood/Air	0	Assumed
Metabolic Parameters		
Liver Vmax	52474 mg/hr	Assumed
Liver KM	237 mg/L	Assumed
Kidney Partition Parameter		
Elimination Partition	0.35	Assumed
Atropine		
Molecular Weight	289 mg/mmol	Heath and McKeown
Partition Coefficients		
Bain	0.67	Assumed
Diaphragm	0.77	Assumed
RPT	0.67	Assumed
Fat	17.6	Assumed
SPT	0.77	Assumed
Thigh	0.77	Assumed

Kidney	1.63	Assumed
Liver	1.53	Assumed
Venous	1	Assumed
Arterial	1	Assumed
Blood/Air	0	Assumed
Metabolic Parameters		
Liver Vmax	52474 mg/hr	Assumed
Liver KM	237 mg/L	Assumed
Kidney Partition Parameter		
Elimination Partition	0.35	Assumed
Acetylcholinesterase		
Molecular Weight	320 mmol/mg	Assumed
Synthesis Rate, X1		
Arterial	0.0001 umol/hr	Gentry et. al.
Brain	0.00002 umol/hr	Scaled from Gentry et. al.
Diaphragm	0.000003 umol/hr	Scaled from Gentry et. al.
RPT	0.00003 umol/hr	Scaled from Gentry et. al.
Fat	0.0 umol/hr	Gentry et. al.
SPT	0.0005 umol/hr	Scaled from Gentry et. al.
Thigh	0.00002 umol/hr	Scaled from Gentry et. al.
Kidney	0.000004 umol/hr	Scaled from Gentry et. al.
Liver	0.00004 umol/hr	Scaled from Gentry et. al.
Venous	0.0001 umol/hr	Gentry et. al.
Initial Concentration		
Arterial	0.001212 umol	Gentry et. al.
Brain	0.04928 umol	Gentry et. al.
Diaphragm	0.000909 umol	Gentry et. al.
RPT	0.008314 umol	Gentry et. al.
Fat	0.0 umol	Gentry et. al.
SPT	0.222196 umol	Gentry et. al.
Thigh	0.011708 umol	Gentry et. al.
Kidney	0.000104 umol	Gentry et. al.
Liver	0.002424 umol	Gentry et. al.
Venous	0.003454 umol	Gentry et. al.
Degradation Constant, X2		
Arterial	0.082508251 hr^{-1}	Calculated
Brain	0.000405844 hr^{-1}	Calculated
Diaphragm	0.00330033 hr^{-1}	Calculated
RPT	0.003608371 hr^{-1}	Calculated
Fat	0.0 hr^{-1}	Calculated
SPT	0.002250266 hr^{-1}	Calculated
Thigh	0.001708234 hr^{-1}	Calculated
Kidney	0.038461538 hr^{-1}	Calculated
Liver	0.01650165 hr^{-1}	Calculated

Venous	0.02895194 hr^{-1}	Calculated
Butyrylcholinesterase		
Molecular Weight	83.3 mmol/mg	Ashani and Pistinner
Synthesis Rate, Y1		
Arterial	0.0001 umol/hr	Gentry et. al.
Brain	0.00002 umol/hr	Scaled from Gentry et. al.
Diaphragm	0.000003 umol/hr	Scaled from Gentry et. al.
RPT	0.00003 umol/hr	Scaled from Gentry et. al.
Fat	0.0 umol/hr	Gentry et. al.
SPT	0.0005 umol/hr	Scaled from Gentry et. al.
Thigh	0.00002 umol/hr	Scaled from Gentry et. al.
Kidney	0.000004 umol/hr	Scaled from Gentry et. al.
Liver	0.00004 umol/hr	Scaled from Gentry et. al.
Venous	0.0001 umol/hr	Gentry et. al.
Initial Concentration		
Arterial	0.00606 umol	Gentry et. al.
Brain	0.016859 umol	Gentry et. al.
Diaphragm	0.002 umol	Gentry et. al.
RPT	0.006236 umol	Scaled from Gentry et. al.
Fat	0.0 umol	Gentry et. al.
SPT	0.190454 umol	Gentry et. al.
Thigh	0.010035 umol	Gentry et. al.
Kidney	0.000782 umol	Gentry et. al.
Liver	0.019392 umol	Gentry et. al.
Venous	0.017271 umol	Gentry et. al.
Degradation Constant, Y2		
Arterial	0.01650165 hr^{-1}	Calculated
Brain	0.00118631 hr^{-1}	Calculated
Diaphragm	0.0015 hr^{-1}	Calculated
RPT	0.004810776 hr^{-1}	Calculated
Fat	0.0 hr^{-1}	Calculated
SPT	0.002625306 hr^{-1}	Calculated
Thigh	0.001993034 hr^{-1}	Calculated
Kidney	0.00511509 hr^{-1}	Calculated
Liver	0.002062706 hr^{-1}	Calculated
Venous	0.005790053 hr^{-1}	Calculated
Carboxylesterase		
Molecular Weight	320 mg/mmol	Assumed
Synthesis Rate, Z1		
Arterial	0.0001 umol/hr	Gentry et. al.
Brain	0.00002 umol/hr	Scaled from Gentry et. al.
Diaphragm	0.000003 umol/hr	Scaled from Gentry et. al.
RPT	0.00003 umol/hr	Scaled from Gentry et. al.

Fat	0.0 umol/hr	Gentry et. al.
SPT	0.0005 umol/hr	Scaled from Gentry et. al.
Thigh	0.00002 umol/hr	Scaled from Gentry et. al.
Kidney	0.000004 umol/hr	Scaled from Gentry et. al.
Liver	0.00004 umol/hr	Scaled from Gentry et. al.
Venous	0.0001 umol/hr	Gentry et. al.
Initial Concentration		
Arterial	5.0904 umol	Gentry et. al.
Brain	0.778104 umol	Gentry et. al.
Diaphragm	0.52722 umol	Gentry et. al.
RPT	442.73754 umol	Scaled from Gentry et. al.
Fat	0.0 umol	Gentry et. al.
SPT	73.007244 umol	Gentry et. al.
Thigh	3.846888 umol	Gentry et. al.
Kidney	4.29957 umol	Gentry et. al.
Liver	110.292 umol	Gentry et. al.
Venous	14.50764 umol	Gentry et. al.
Degradation Constant, Z2		
Arterial	1.96448 * 10-5 hr^{-1}	Calculated
Brain	2.57035 * 10-5 hr^{-1}	Calculated
Diaphragm	5.69022 * 10-6 hr^{-1}	Calculated
RPT	6.77602 * 10-8 hr^{-1}	Calculated
Fat	0.0 hr^{-1}	Calculated
SPT	6.848864 * 10-6 hr^{-1}	Calculated
Thigh	5.19901 * 10-6 hr^{-1}	Calculated
Kidney	9.30326 * 10-7 hr^{-1}	Calculated
Liver	3.626674 * 10-7 hr^{-1}	Calculated
Venous	6.89292 * 10-6 hr^{-1}	Calculated
Acetylcholine		
Molecular Weight	146 mg/mmol	about.com

Appendix C. Test Protocols and Results

Table 3. Series A, B, and C Test Protocols

Test No.	
Time-based dosing of antidotes	
1	2 mg of atropine and 600 mg of oximes every 5 minutes starting when symptoms equal to 1.09
2	2 mg of atropine every 5 minutes starting when symptoms equal to 1.09
3	600 mg of oximes every 5 minutes starting when symptoms equal to 1.09
Symptoms-based dosing of antidotes	
4	2 mg of atropine and 600 mg of oximes every time symptoms reach 1.09*
5	2 mg of atropine every time symptoms reach 1.09*
6	600 mg of oximes every time symptoms reach 1.09*
Higher initial dose of antidotes; followed by time-based dosing of antidotes	
7	6 mg of atropine and 1800 mg of oximes when symptoms reach 1.09; additional 2 mg of atropine and 600 mg of oximes every 5 minutes
8	6 mg of atropine when symptoms reach 1.09; additional 2 mg of atropine every 5 minutes
9	1800 mg of oximes when symptoms reach 1.09; additional 600 mg of oxime every 5 minutes
Higher initial dose of antidotes; followed by symptoms-based dosing of antidotes	
10	6 mg of atropine and 1800 mg of oximes when symptoms reach 1.09; additional 2 mg of atropine and 600 mg of oximes every time symptoms reach 1.09*
11	6 mg of atropine when symptoms reach 1.09; additional 2 mg of atropine every time symptoms reach 1.09*
12	1800 mg of oximes when symptoms reach 1.09; additional 600 mg of oximes every time symptoms reach 1.09*
Pretreatment of antidotes by 5 minutes; followed by time-based doses of antidotes	
13	2 mg of atropine and 600 mg of oximes 5 minutes before organophosphate exposure begins and repeated every 5 minutes
14	2 mg of atropine administered 5 minutes before organophosphate exposure begins and repeated every 5 minutes
15	600 mg of oximes administered 5 minutes before organophosphate exposure begins and repeated every 5 minutes.
Pretreatment of antidotes by 5 minutes; followed by symptoms-based doses of antidotes	
16	2 mg of atropine and 600 mg of oximes 5 minutes before organophosphate exposure begins and repeated every time symptoms reach 1.09*
17	2 mg of atropine 5 minutes before organophosphate exposure begins and repeated every time symptoms reach 1.09*
18	600 mg of oximes administered 5 minutes before organophosphate exposure begins and repeated every time symptoms reach 1.09*
Control	
19	No administration of antidotes.

*not to exceed injections more frequent than 5 minute intervals

Reaction rate coefficients for Series A tests: $L_1 = 40{,}000$; $L_2 = 100$; $L_3 = 10{,}000$; $M_1 = 80{,}000$; $M_2 = 100$; $M_3 = 10{,}000$; $N_1 = 20{,}000$; $N_2 = 100$; $N_3 = 10{,}000$; $O_1 = P_1 = R_1 = 50$

Reaction rate coefficients for Series B and C tests: $L_1 = 20{,}000$; $L_2 = 50$; $L_3 = 20{,}000$; $M_1 = 40{,}000$; $M_2 = 50$; $M_3 = 20{,}000$; $N_1 = 10{,}000$; $N_2 = 50$; $N_3 = 10{,}000$; $O_1 = P_1 = R_1 = 100$

Table 4. Series A Test Results

Test No.	First appearance of symptoms (min)	Time at death (min)	Total amount of atropine (mg)	Total amount of oximes (mg)	No. of atropine injections	No. of oxime injections	Total time symptoms > 1.09 (min)	Total time symptoms > 1.16 (min)	Total time symptoms < 0.91 (min)	Total time symptoms < 0.84 (min)	Symptom Levels at 3 hours	Symptoms trend at 3 hours
1	25.2	88.2	62	18576	31	31	8.4	0	114.0	102.0	0.43	Dec
2	25.2	87.6	62	0	31	0	8.4	0	114.0	102.0	0.43	Dec
3	25.2	45.6	0	18576	0	31	154.8	144.6	0	0	1.72	Inc
4	25.2	N/A	4	1200	2	2	8.4	0	0	0	0.98	Dec
5	25.2	N/A	4	0	2	0	0	0	0	0	0.98	Dec
6	25.2	45.6	0	18576	0	31	0	144.6	0	0	1.72	Inc
7	25.2	80.4	66	19776	31	31	144.6	0	121.8	109.2	0.41	Dec
8	25.2	80.4	66	0	31	0	0	0	121.8	109.2	0.41	Dec
9	25.2	45.6	0	19776	0	31	154.8	144.6	0	0	1.72	Inc
10	25.2	108.0	6	18000	1	1	2.4	0	90.6	15.0	0.99	Inc
11	25.2	108.0	6	0	1	0	2.4	0	90.6	15.0	0.99	Inc
12	25.2	45.6	0	19776	0	31	154.8	144.6	0	0	1.72	Inc
13	12.6	36.0	72	21600	36	36	0	0	167.4	154.2	0.33	Dec
14	12.6	36.0	72	0	36	0	0	0	167.4	154.2	0.33	Dec
15	25.8	45.6	0	21600	0	36	154.8	144.6	0	0	1.72	Inc
16	92.4	N/A	4	1200	2	2	4.2	0	0	0	0.97	Inc
17	94.4	N/A	4	0	2	0	4.2	0	0	0	0.97	Inc
18	25.2	45.6	0	19200	0	32	154.8	144.6	0	0	1.72	Inc
19	25.2	45.6	0	0	0	0	154.8	144.6	0	0	1.72	Inc

Table 5. Series B and C Test Results

Test No.	First appearance of symptoms (min)	Time at death (min)	Total amount of atropine (mg)	Total amount of oximes (mg)	No. of atropine injections	No. of oxime injections	Total time symptoms > 1.09 (min)	Total time symptoms > 1.16 (min)	Total time symptoms < 0.91 (min)	Total time symptoms < 0.84 (min)	Symptom Levels at 3 hours	Symptoms trend at 3 hours
1	37.8	80.4	56	16800	28	28	2.4	0	117.6	109.8	0.30	Dec
2	37.8	80.4	56	0	28	0	2.4	0	117.6	109.8	0.30	Dec
3	37.8	73.2	0	16800	0	28	142.2	117.0	0	0	1.31	Inc
4	37.8	N/A	2	600	1	1	2.4	0	0	0	1.04	Inc
5	37.8	N/A	2	0	1	0	2.4	0	0	0	1.04	Inc
6	37.8	73.2	0	16800	0	28	142.2	117.0	0	0	1.31	Inc
7	37.8	76.8	60	18000	28	28	1.2	0	113.4	113.4	0.28	Dec
8	37.8	76.8	60	0	28	0	1.2	0	113.4	113.4	0.28	Dec
9	37.8	72.6	0	18000	0	28	142.2	117.0	0	0	1.31	Inc
10	37.8	73.2	6	18000	1	1	1.2	0	106.8	106.8	0.80	Inc
11	37.8	83.4	6	0	1	0	1.2	0	106.8	106.8	0.80	Inc
12	37.8	73.2	0	18000	0	28	142.2	117.0	0	0	1.31	Inc
13	12.6	30.6	72	21600	36	36	0	0	167.4	159.6	0.21	Dec
14	12.6	30.6	72	0	36	0	0	0	167.4	159.6	0.21	Dec
15	37.8	73.2	0	21600	0	36	142.2	117.0	0	0	1.31	Inc
16	14.4	N/A	4	1200	2	2	1.2	0	81.6	0	1.06	Dec
17	14.4	N/A	4	0	2	0	1.8	0	81.6	0	1.06	Dec
18	37.8	73.2	0	17400	0	28	142.2	117.0	0	0	1.31	Inc
19	37.8	73.2	0	0	0	0	136.2	117.0	0	0	1.31	Inc

Table 6. Series D and E Test Protocol

Test No.	L_1	L_2	L_3	O_1	Oxime Dose
1	20000	50	20000	100	No dose
2	20000	50	20000	100	600 mg of oxime every 5 min starting at time 0 and ending after 30 min
3	20000	50	20000	500	No dose
4	20000	50	20000	500	600 mg of oxime every 5 min starting at time 0 and ending after 30 min
5	20000	50	5000	100	No dose
6	20000	50	5000	100	600 mg of oxime every 5 min starting at time 0 and ending after 30 min
7	5000	50	5000	7000	No dose
8	5000	50	5000	7000	2 doses of 600 mg of oxime at time 0 and at time 5 min
9	20000	50	100	2500	No dose
10	20000	50	100	2500	2 doses of 600 mg of oxime at time 0 and at time 5 min

$M_1 = 2 * L_1$
$N_1 = 0.5 * L_1$
$L_2 = M_2 = N_2$
$O_1 = P_1 = R_1$

Table 7. Series D Test Results

Test No.	First appearance of symptoms (min)	Time at death (min)	Total amount of oximes (mg)	No. of oxime injections	Total time symptoms > 1.09 (min)	Total time symptoms > 1.16 (min)	Symptom levels at 3 hours	Symptoms trend at 3 hours	Min AChE levels (mg)	Time at min AChE levels (min)
1	37.8	73.2	0	0	142.2	117	1.31	Inc	0.011677	26.4
2	37.8	73.2	3600	6	142.2	117	1.31	Inc	0.011678	29.4
3	37.8	73.2	0	0	142.2	117	1.31	Inc	0.011677	26.4
4	37.8	73.8	3600	6	142.2	116.4	1.30	Inc	0.011686	26.4
5	37.8	73.8	0	0	142.2	117.6	1.30	Inc	0.011696	27.0
6	37.8	73.8	3600	6	142.2	117.6	1.30	Inc	0.011703	27.0
7	N/A	N/A	0	0	0	0	1.09	Inc	0.014369	27.6
8	N/A	N/A	1200	2	0	0	1.08	Inc	0.014484	25.8
9	48.0	103.2	0	0	132	87	1.22	Inc	0.012662	27.0
10	154.2	N/A	1200	2	25.8	0	1.09	Inc	0.014307	27.0

Table 8. Series E Test Results

Test No.	First appearance of symptoms (min)	Time at death (min)	Total amount of oximes (mg)	No. of oxime injections	Total time symptoms > 1.09 (min)	Total time symptoms > 1.16 (min)	Symptom levels at 3 hours	Symptoms trend at 3 hours	Min AChE levels (mg)	Time at min AChE levels (min)
1	37.8	73.2	0	0	142.2	117	1.31	Inc	0.011677	26.4
2	37.8	73.2	3600	6	142.2	117	1.31	Inc	0.011678	28.8
3	37.8	73.2	0	0	142.2	117	1.31	Inc	0.011677	26.4
4	37.8	73.8	3600	6	142.2	116.4	1.30	Inc	0.011683	27.0
5	37.8	73.8	0	0	142.2	116.4	1.30	Inc	0.011690	27.0
6	37.8	73.8	3600	6	142.2	116.4	1.30	Inc	0.011701	27.0
7	N/A	N/A	0	0	0	0	1.09	Inc	0.014369	27.6
8	N/A	N/A	1200	2	0	0	1.08	Inc	0.014474	26.4
9	48.0	103.2	0	0	132.0	87.0	1.22	Inc	0.012662	27.0
10	102.6	N/A	1200	2	77.4	0	1.11	Inc	0.014044	25.8

Table 9. Series F Test Protocol

	Fast OP reaction	Slow OP reaction
Fast aging	10 minute aging half-life L1 = 1000000 L3 = 4.5 Tests 1-3, 19-21	10 minute aging half-life L1 = 50000 L3 = 4.5 Tests 4-6, 22-24
Moderate aging	5 hour aging half-life L1 = 1000000 L3 = 0.16 Tests 7-9, 25-27	5 hour aging half-life L1 = 50000 L3 = 0.16 Tests 10-12, 28-30
Slow aging	48 hour aging half-life L1 = 1000000 L3 = 0.014 Tests 13-15, 31-33	48 our aging half-life L1 =50000 L3 = 0.014 Tests 16-18, 34-36

Each scenario consisted of 3 tests. For the first test, no oxime administration. For the second tests, 600 mg of oxime repeated every 5 minutes, starting at noticeable symptoms. For the third test, 600 mg of oxime repeated every 5 minutes, starting at time 0.

Tests 1 through 18 used an exposure of 5 mg of OP for 15 minutes starting at time +5 minutes

Tests 19 through 36 used an exposure of 10 mg of OP for 20 minutes starting at time +5 minutes

Oxime reaction rate coefficient set equal to 20

Table 10. Series F Test Results

Test No.	First appearance of symptoms (min)	Time at death (min)	Total amount of oxime (mg)	No of oxime injections	Time symptoms >1.09	Time symptoms > 1.16 (min)	Symptom levels at 3 hours	Symptoms trend at 3 hours	Min AChE level (mg)	AChE level at 3 hours (mg)
1	26.4	0.76	0	0	153.6	144.6	1.78	Inc	0.007683	0.007691
2	26.4	0.76	18000	30	153.6	144.6	1.87	Inc	0.007007	0.007031
3	25.8	0.73	21600	36	154.2	146.4	1.99	Inc	0.006309	0.006375
4	27.6	0.83	0	0	152.4	140.4	1.57	Inc	0.009209	0.009216
5	26.0	0.83	18000	30	152.4	140.4	1.53	Inc	0.009209	0.009607
6	29.4	0.94	21600	36	150.6	133.8	1.39	Inc	0.009888	0.010821
7	26.4	0.76	0	0	153.6	144.6	1.78	Inc	0.007683	0.007691
8	26.4	0.76	18000	30	153.6	147.6	1.71	Dec	0.005543	0.010338
9	25.2	0.71	21600	36	154.8	147.6	1.59	Dec	0.005585	0.011114
10	27.6	0.83	0	0	152.4	130.2	1.57	Inc	0.009209	0.009216
11	27.6	0.85	18000	30	152.4	70.8	1.09	Dec	0.009209	0.014967
12	30.6	N/A	21600	36	70.8	0	1.05	Dec	0.010160	0.015298
13	26.4	0.76	0	0	2.56	144.6	1.78	Inc	0.007683	0.007691
14	26.4	0.76	18000	30	153.6	144.6	1.65	Dec	0.005497	0.011356
15	25.2	0.71	21600	36	153.6	147.6	1.53	Dec	0.005563	0.012229
16	27.6	0.83	0	0	152.4	140.4	1.57	Inc	0.009209	0.009216
17	27.6	0.85	13200	22	111.0	57.6	1.05	Dec	0.009209	0.015695
18	30.6	N/A	21600	36	58.2	0	1.02	Dec	0.010172	0.015727
19	18.0	32.4	0	0	162.0	157.8	3.79	Inc	0.000001	0.000016
20	18.0	32.4	19200	32	162.0	157.8	3.38	Inc	0.000040	0.001126
21	18.0	32.4	21600	36	162.0	157.8	3.28	Inc	0.001334	0.001411
22	21.0	36.6	0	0	159.0	153.6	2.76	Inc	0.002929	0.002941
23	21.0	36.6	18000	30	159.0	153.6	2.38	Inc	0.003193	0.004518
24	21.6	37.8	21600	36	158.4	152.4	2.03	Inc	0.004586	0.006272
25	18.0	32.4	0	0	162.0	157.8	3.79	Inc	0.000001	0.000016
26	18.0	32.4	19200	32	162.0	157.8	1.88	Dec	0.000058	0.010069
27	18.0	32.4	21600	36	162.0	157.8	1.79	Dec	0.003070	0.010504
28	21.0	36.6	0	0	159.0	153.6	2.76	Inc	0.002929	0.002941
29	21.0	36.6	18000	30	159.0	151.8	1.16	Dec	0.003374	0.014468
30	21.6	0.65	21600	36	158.4	88.2	1.10	Dec	0.005547	0.014876
31	18.0	39.0	0	0	162.0	157.8	3.79	Inc	0.000001	0.000016
32	18.0	32.4	19200	32	162.0	157.8	1.76	Dec	0.000058	0.011536
33	18.0	32.4	21600	36	162.0	157.8	1.67	Dec	0.003158	0.011970
34	21.0	36.6	0	0	159.0	153.6	2.76	Inc	0.002929	0.002941
35	21.0	36.6	18000	30	151.8	109.2	1.08	Dec	0.003382	0.015650
36	21.6	39.0	21600	36	114.6	70.2	1.05	Dec	0.005593	0.015689

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14. ABSTRACT

The National Preparedness Vision requires the U.S. be prepared to prevent, protect against, respond to, and recover from all hazards associated with a chemical attack. Results of this study demonstrate that we cannot protect service members and first responders as required following a nerve agent attack. The research presented herein aimed to construct a physiologically based pharmacokinetic model to determine optimal therapeutic strategies for organophosphate (nerve agent) poisoning. The constructed model integrated organophosphates and two antidotes, atropine and oximes. Model results reasonably mirrored literature data and anecdotal observations of organophosphate poisoning. Results suggest a symptoms-based dosing strategy of atropine and a time-based dosing strategy of oximes. For patients severely poisoned with organophosphorus nerve agents, model results support documented claims of oxime's inefficacy and tendency to heighten the severity of poisoning. The results strongly indicate that military personnel attacked with nerve agents are at a significant health risk if they employ their prescribed treatment as current doctrine dictates. Results presented herein suggest that oxime use be discontinued as currently prescribed within the context of nerve agent exposure; its use will not alter the effects of nerve agent exposure and may increase the adverse effects.

15. SUBJECT TERMS
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