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Sarin (GB, O-isopropyl methylphosphonofluoridate) neurotoxicity: critical review

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Abstract

Sarin (GB, *O*-isopropyl methylphosphonofluoridate) is a potent organophosphorus (OP) nerve agent that inhibits acetylcholinesterase (AChE) irreversibly. The subsequent build-up of acetylcholine (ACh) in the central nervous system (CNS) provokes seizures and, at sufficient doses, centrally-mediated respiratory arrest. Accumulation of ACh at peripheral autonomic synapses leads to peripheral signs of intoxication and overstimulation of the muscarinic and nicotinic receptors, which is described as "cholinergic crisis" (i.e. diarrhea, sweating, salivation, miosis, bronchoconstriction). Exposure to high doses of sarin can result in tremors, seizures, and hypothermia. More seriously, build-up of ACh at neuromuscular junctions also can cause paralysis and ultimately peripherally-mediated respiratory arrest which can lead to death via respiratory failure. In addition to its primary action on the cholinergic system, sarin possesses other indirect effects. These involve the activation of several neurotransmitters including gamma-amino-butyric acid (GABA) and the alteration of other signaling systems such as ion channels, cell adhesion molecules, and inflammatory regulators. Sarin exposure is associated with symptoms of organophosphate-induced delayed neurotoxicity (OPIDN) and organophosphate-induced chronic neurotoxicity (OPICN). Moreover, sarin has been involved in toxic and immunotoxic effects as well as organophosphate-induced endocrine disruption (OPIED). The standard treatment for sarin-like nerve agent exposure is post-exposure injection of atropine, a muscarinic receptor antagonist, accompanied by an oxime, an AChE reactivator, and diazepam.

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Declaration of interest

This manuscript was prepared by the authors who are affiliated with Duke University, aimed at engaging scientists from academia, government, industry, research institutes, and the public to identify and resolve health and environmental issues related to OP compounds and sarin exposure in particular. This is a significant issue considering that an estimated 200 000 American military personnel were suspected to have been exposed to low-level sarin that was released following the destruction of Iraqi weapon stockpile in Khamisiyah, Iraq in 1991. Many of these soldiers still complain of symptoms of the Gulf War illness. Recent attacks against civilian populations by terrorists ranging from fanatic religious cult members in Japan and dictators in Iraq and Syria have resulted in the death of thousands of people, including children. The authors have sole responsibility for this paper that reflects their views and of the writing and content of the paper. The views and opinions expressed in the paper do not necessarily reflect the views of the author's employers. Mention of trade names does not constitute endorsement. The senior author, MBA investigated health effects of sarin through the Department of Defence grants. He testified of its involvement of chemical exposure, including sarin in the Gulf War illness before the U.S. Senate Veterans' Committee and presented his research in DOD meetings. None of the authors has recently or is currently been involved as an expert witness in litigation on the subject of this paper. The authors declare that there is no conflict of interest.

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Keywords

Neurotoxicity; organophosphates; sarin; toxicity; oximes; OPIDN; OPICN; AChE; NTE; BChE; LD50

Introduction

Sarin (GB, O-isopropyl methylphosphonofluoridate) is a potent organophosphorus (OP) nerve agent that inhibits acetylcholinesterase (AChE) irreversibly. Accumulation of acetylcholine (Ach) at peripheral autonomic synapses leads to peripheral signs of intoxication and overstimulation of the muscarinic and nicotinic receptors, which is described as "cholinergic crisis" (i.e. diarrhea, sweating, salivation, miosis, and bronchoconstriction). Exposure to high doses of sarin can result in tremors, seizures, and hypothermia. A more severe effect of sarin is the build-up of ACh in the central nervous system (CNS) which causes paralysis and ultimately peripherally-mediated respiratory arrest, leading to death. Sarin was first synthesized as a potential insecticide in 1938 by German scientists (Brown and Brix 1998). During World War II, sarin was later developed into a chemical warfare agent along with cyclosarin (GF, cyclohexyl methylphosphonofluoridate), tabun (GA, ethyl dimethylphosphoramido-cyanidate), soman (GD, O-pinacolyl methylphosphonofluoridate), and VX (O-ethyl S-[2-(diisopropylamino)ethyl] methylphosphonothioate) (Figure 1, Abu-Qare & Abou-Donia 2002). The recent use of sarin in the Middle East against both military and civilian targets and by terror groups in Japan highlighted the threat of chemical warfare agents against civilian populations. Not only is the civilian population at risk of exposure to sarin that is used by the military as a nerve agent, but it is also at risk of exposure to OP pesticides that are readily available worldwide. Approximately 2.5 million pesticide poisonings occur annually, most of which result from OP pesticides, and cause 250 000 deaths each year (WHO 1990). Numerous studies of sarin have been undertaken, and a survey of publications on sarin research cited in ScienceDirect online (as of June 2016) revealed the following statistics: total number of sarin publications: 14 339; analysis: 10 063; cholinergic neurotoxicity: 1085; delayed neurotoxicity: 412; chronic neurotoxicity: 1314; endocrine disruption: 182.

The scope of this article is to present a critical review of the development of sarin, its wide-spread use, its regulations, and its toxicity. This article also focuses on various strategies used to treat the four stages of sarin induced-neurotoxicity: (1) acute cholinergic toxicity, (2) organophosphate-induced delayed neurotoxicity (OPIDN), (3) organophosphate-induced chronic neurotoxicity (OPICN), and (4) organophosphate-induced endocrine disruption (OPIED). Finally, this article will identify, for non-specialist toxicologists, the damage that sarin can cause to the nervous system and its long-term neurobehavioral effects. Table 1 lists all of the abbreviations, acronyms, and symbols cited in this review. Table 2 lists the IUPAC nomenclature, CAS No., and molecular formula of chemicals cited in the text.

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Chemical weapons

World War I witnessed the dawn of the modern use of chemical weapons by both sides of the conflict. Such weapons mostly consisted of chlorine, phosgene, and mustard gas and resulted in approximately 100 000 deaths. As many as one million casualties world-wide attributed to chemical weapons have occurred since World War I. During the Cold War period (1947–1991), significant development, manufacturing, and stockpiling of chemical weapons took place (Table 3). In the early 1950s, sarin was a standard chemical weapon of both the North Atlantic Treaty Organization (NATO) and the Union of Soviet Socialist Republics (USSR), but by the 1960s, these countries had agreed to destroy their stockpiles of sarin (Rosman et al. 2014). The public concern regarding these dangerous chemicals led to worldwide regulations and control of chemical weapons through the following major agreements (Somani & Romano, 2001; Abraham et al. 2002).

Geneva Protocol for chemical weapons (1925): This agreement prohibited the use of asphyxiating, poisonous or other gases, and chemical and bacteriological methods of warfare. It did not prohibit the development, production, or stockpiling of chemical weapons.

Chemical Weapons Convention (CWC, 1997): The official name of this treaty is the "Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on their Destruction." CWC is administered by the Organization of the Prohibition of Chemical Weapons (OPCW) that is based in The Hague, Netherlands. A total of 72 524 metric tons of chemical agent, 8.67 million chemical munitions, and 97 production facilities have been declared to OPCW (Pitschmann 2014). As of October 2015, a total of 65 720 of the 72 524 (90%) metric tons of chemical agent have been verifiably destroyed. More than 57% (4.97 million) of the chemical munitions and containers have been destroyed. The OPCW was awarded the Nobel Peace Prize in 2013 for its work in controlling chemical weapons (Üzümcü 2014).

Incidences of human exposure to sarin

Even though the use of chemical weapons was banned by the Geneva Protocol (1925) and the development, production, stockpiling, and use of chemical weapons by the CWC (1997), nerve agents have been used repeatedly both by terrorist groups and governments (Ruhl et al. 1994). Nerve agents, including sarin, were used by the Iraqi government on both the Iranian army and the Iraqi Kurds in the 1980s (Abraham et al. 2002, Table 4). Although sarin was never used against the Allied Forces during the Gulf War of 1991–1992, on 10 March 1991, an estimated 200 000 American military personnel were exposed to low-level sarin following the destruction of an Iraqi ammunition dump, in Khamisiyah, Iraq, that contained sarin (Table 4). This exposure to sarin may have contributed to Gulf War veterans' illnesses characterized by memory deficits, cognition problems, muscle weakness, and joint pain (IOM 1995).

Additionally, sarin was used in 1994 in Matsumoto, Japan (Morita et al., 1995) and in 1995 in Tokyo (Masuda et al., 1995) in subway attacks by the "Aum Supreme Truth" cult (Yanagisawa et al. 2006). The Matsumoto attack resulted in approximately 600 exposures to

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sarin and eight deaths (Table 4). The Tokyo attack resulted in approximately 5500 exposures to sarin and 12 deaths. While many of the deaths resulting from sarin exposure occurred instantaneously, victims continue to die up to three months following the attacks (Yokoyama et al. 1998; Araki et al. 2005).

The most recent illegal use of sarin took place in the early hours of 21 August 2013 when the Ghouta section of Damascus was subjected to a sarin attack during the Syrian Civil War (Rosman et al. 2014). This sarin attack resulted in 3600 exposures and approximately 1729 deaths (Table 4).

Synthesis and chemical properties of sarin

Sarin is synthesized by reacting methylphosphonyl difluoride with a mixture of isopropyl amine and isopropyl alcohol (Figure 2). Sarin contains a single chiral center and is generally synthesized to form a pair of enantiomers (Li et al. 2001; Abu-Qare & Abou-Donia 2002). While this synthesis creates a racemic mixture of the sarin enantiomers, the Sp-enantiomer causes a higher degree of acute toxicity because it inactivates AChE at approximately 10 times the rate of the Rp-enantiomer (Li et al. 2001). A by-product of the sarin synthetic process is O,O-di-isopropyl methylphosphonate (DIMP), a less potent inhibitor of AChE than sarin (Bucci et al. 2003).

Chemical properties of sarin

Sarin is classified as an OP nerve agent with a molecular weight of 140.1 g/mol (Leikin et al. 2002). Like the other nerve agents, sarin is a phosphonate and contains a fluorine atom as its leaving group (Figure 2). At room temperature, sarin exists as a clear, colorless, odorless liquid with a boiling point of 147 °C (Table 5). While sarin is miscible with water, when these two liquids are mixed, sarin is hydrolyzed to a much less toxic hydrolysis product at room temperature. At neutral pH and room temperature, sarin has a half-life of 5.4 h. The rate of the sarin hydrolysis reaction increases with increasing pH, such that its half-life in alkaline conditions, at a pH of 9, is only 15 min.

Sarin (GB) is the most highly volatile of the nerve agents: tabun (GA), cyclosarin (GF), soman (GD), and VX (Leikin et al. 2002). Due to its high volatility of 22 000 mg/m^3 at 25 °C, it is easily converted from liquid to gaseous form during deployment. When utilized for chemical warfare, it is either vaporized from the heat generated by a weapon or aerosolized by a sprayer. As such, the most common and simultaneously lethal route of exposure is inhalational (Cannard 2006). The inhalational dose is measured in units of mg-min/m^3; for human exposure to sarin vapor, the total dose is the product of vapor concentration in mg/m^3 and the concentration–time (*t*), results in an LCt_{50} of 100 mg-min/m^3 (Cannard 2006). The LD_{50} values of sarin in humans and other animal species following various routes of exposure are listed in Table 6.

Metabolism and pharmacokinetics of sarin

Although sarin is extremely toxic, it is short-lived inside the body. Once sarin enters the body, it is quickly metabolized and excreted. The primary metabolite is a pharmacologically inactive hydrolysis product, isopropyl methylphosphonic acid (Shih et al. 1994). This

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metabolite is rapidly excreted, primarily through the urine. In rats subcutaneously injected with sarin, 59% and 91% of a given dose was excreted after 4 and 24 h, respectively. After two days, sarin metabolite was undetectable in the urine. Based on this result, it was calculated that sarin has a terminal half-life of 3.7 ± 0.1 h in the urine of rats. Other pharmacokinetic studies have been performed in guinea pigs which some consider a highly predictive model for human exposure (Dawson 1994). It was shown that $0.1 \times LC_{50}$ of sarin in guinea pigs exhibited a half-life of 56.39 and 923 min in the plasma and red blood cells (RBCs), respectively (Whalley et al. 2007).

Sarin-induced cholinergic neurotoxicity

Mechanisms of sarin-induced cholinergic neurotoxicity

The nerve agent sarin was tailor-made to kill animals including humans by disrupting the nervous system via inhibition of AChE, an enzyme essential for life. AChE functions to hydrolyze the neurotransmitter ACh at ACh receptors (AChRs). Inhibition of the enzyme results in the disruption of the cholinergic system and may lead to death.

Cholinergic system

AChE is present throughout the CNS and the peripheral nervous system (PNS). The CSN consists of the brain and the spinal cord, and the PNS contains ganglia and nerve fibers that connect the CNS to the body (Karalliedde et al. 2000). The sympathetic nervous system (SNS) is normally active at all times. It accelerates heart rate, blood pressure, and blood sugar, widens bronchioles and pupils, and increases secretion of epinephrine from the adrenal medulla. The parasympathetic nervous system is concerned primarily with the conservation and restoration of energy. It slows heart rate, lowers blood pressure, stimulates gastrointestinal movements and secretion, protects the eyes from excessive light, and empties the urinary bladder and the rectum. Sarin-induced inhibition of AChE exaggerates all of these functions. This inhibition results initially in excitation followed by paralysis of cholinergic transmission because of the accumulation of ACh at both muscarinic and nicotinic receptors in the CNS and PNS. Inhibition of various types of these receptors results in the specific loss of function related to the location of each receptor in each specific organ.

Muscarinic acetylcholine receptors (mAChRs)

Muscarinic receptors are found primarily on smooth muscles and exocrine glands controlled by the PNS. Sarin-inhibition of the muscarinic AChRs generally results in excessive secretions and smooth muscle contractions. The mAChRs consist of five sub-types: M1, M2, M3, M4, and M5. The subtype muscarinic receptors are distributed as follows: M1 (brain [cortex, hippocampus], glands, and sympathetic ganglia); M2 (heart, hind-brain, and smooth muscle); M3 (smooth muscle, vasculature endothelium, secretory glands, heart, and brain); M4 (brain [forebrain and striatum]); M5 (brain [substantia nigra] and eye).

Nicotinic acetylcholine receptors (nAChRs)

The subtype nicotinic receptors are distributed as follows: nicotinic skeletal muscle (NM); $(\alpha 1)2\beta 1\varepsilon\delta$ at neuromuscular junction (skeletal muscle contraction); neuronal peripheral (NN); $(\alpha 3)2(\beta 4)$ at autonomic ganglia and adrenal medulla (secretion of catecholamine);

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neuronal central (CNS); (α4)2(β4)3 at pre- and post-junctional sites; (α7)5 pre- and post-synaptic sites. Nicotinic receptors are found on the autonomic ganglia connecting the pre- and post-ganglinic neurons as well as the neuromuscular junctions, whereas overstimulation of the nicotinic receptors causes muscle twitching (Karalliedde et al. 2000).

Acetylcholinesterase

The three-dimensional structure of AChE reveals the active center to be located at the base of a narrow gorge about 20 Å in depth (Sussman et al. 1991). The active center (Figure 3) includes the following four sites: the catalytic triad (GLU334, HIS447, SER203), the acetyl pocket (Phe295, Phe297), the choline subunit (Trp 86, Glu 202, Tyr 337), and the peripheral site (Trp 286, Tyr 72, Tyr 124, ASP 74) (Abou-Donia 2003).

Acetylcholine

ACh, the natural substrate of AChE, is hydrolyzed by the serine hydroxyl in the catalytic triad of AChE that is rendered highly nucleophilic through the formation of hydrogen bonds between imidazole group with histidine and the glutamate carboxyl group and the imidazole group and the hydroxyl of the serine (Cannard 2006). This system creates a partial negative charge on the hydroxyl group oxygen that may attach to the partially positively charged carbon of the carboxyl of ACh or phosphoryl of organophosphate inhibitors. The acetyl enzyme is rapidly hydrolyzed to yield acetate and the active AChE. This enzyme has the capacity to hydrolyze 6×10^5 ACh molecules per molecule enzyme per minute, indicating a turnover time of 150 μs (Figure 4).

Sarin inhibition of AChE

Sarin inhibits AChE by phosphorylating the serine hydroxyl group at the catalytic triad site (Abou-Donia 1981, 2003; Karalliedde et al. 2000; Abdel-Rahman et al. 2002; Figure 3). If the phosphorylated enzyme contains a methyl- or ethyl group, the enzyme is regenerated in several hours by hydrolysis. On the other hand, virtually no hydrolysis occurs with the isopropyl group (e.g. sarin) because phosphorylated AChE undergoes aging, a process that involves the loss of an alkyl group, resulting in a negatively charged monoalkyl enzyme and a stabilized complex (Karalliedde et al. 2000; Figure 5). After aging has occurred, the dephosphorylating reaction cannot occur, and the only way to recover the AChE activity is through the synthesis of new enzymes (Abou-Donia 2003; Newmark 2007). The half-life of the sarin-bound AChE complex before aging is about 3 h (Vale et al. 2011).

Symptoms and signs of sarin-induced cholinergic neurotoxicity

Cholinergic neurotoxicity of sarin in humans

Much of the current knowledge of sarin's effects on humans is based on the clinical records of the victims of the 1994 Matsumoto subway attack. Immediately after the Matsumoto sarin attack, muscarinic symptoms were observed in victims, and they included miosis, ocular pain, blurred vision, rhinorrhea, headache, dyspnea, cough, salivation, and diarrhea. Three weeks later, most of the affected persons had recovered (Yanagisawa et al. 2006). On the other hand, patients with the nicotinic symptoms of muscular twitching and weakness were not common; these symptoms were only present in critically to severely affected patients.

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Loss of consciousness and generalized convulsion were present acutely in critically affected patients, but disappeared within a week. In patients who had marked miosis (pupil diameter below 1.0 mm in 21 cases and moderate miosis [1.0–2.0 mm] in 93 cases), serum butyl cholinesterase (BChE) activity was decreased 10% and 20%, respectively (Yanagisawa et al. 2006). Patients with no miosis had no decrease in BChE activity. Miosis usually disappeared within one month. Erythrocyte AChE was reduced in 20 patients out of 58 seen within three weeks. The following signs and symptoms were also observed in these patients: agitation, conjunctival inflammation, dim and blurred vision, eye pain, miosis, tearing, chest oppression, cough, dyspnea, wheezing, tachypnea, diarrhea, nausea, vomiting, convulsion, decreased consciousness, and dizziness (Okumura et al. 1996). The gastrointestinal symptoms that were observed with sarin exposure were likely due to the effects of sarin on AChE in the gastrointestinal system (Chanda et al. 2010). Furthermore, a study of asymptomatic rescue workers of the Tokyo subway attack, who had experienced low-level sarin exposure, revealed a chronic decline of memory function when they presented three to four years after this low-level exposure (Nishiwaki et al. 2001). It was suggested that the mechanism of memory disturbance might be a consequence of lasting hippocampal pathology induced by sarin.

Sarin-induced death in humans

Acute exposure of humans to organophosphate causes death within 24 h in untreated cases and within 10 days in treated cases (Namba et al. 1971). Death usually results from a combination of excessive bronchial secretion, pulmonary edema, bronchospasm, bronchoconstriction, central apnea, and respiratory muscle paralysis of the respiratory center in the medulla (Newmark 2007). This is caused by sarin's interaction with cholinergic components of the respiratory centers in the CNS that are essential to breathing regulation. These groups include the dorsolateral nucleus tractus soltarius, ventrolateral medulla, and the pneumotaxic center (Carey et al. 2013). Sarin also increases glycine and gamma-amino-butyric acid (GABA) production causing respiratory rate depression and reduced phrenic nerve activity (Carey et al. 2013). The inhalation of sarin at levels close to LC_{50} can cause increased levels of hypoxia-induced factor-1α, bronchoconstriction, and pro-inflammatory cytokine surges, which alter the lung epithelium, increase bronchosecretions, and can lead to death (Carey et al. 2013; Gundavarapu et al. 2014). Studies showed that acute sarin poisoning causes changes to the oxidative homeostasis and biochemical markers in rats intramuscularly injected with 50% of sarin LD_{50} (Pohanka et al. 2012; Abou-Qare and Abou-Donia 2001b).

Cholinergic neurotoxicity of sarin in animals

Effect of sarin on brain AChE activity

Animal studies have also contributed to current knowledge on the effects of sarin in human beings. Moser (2007) evaluated the current literature on animal studies of chronic OP compound exposure and found that the results of these animal studies are appropriate to extrapolate to humans. The i.m. LD_{50} for sarin was determined to be 100 μg/kg in male Sprague–Dawley (SD) rats (Abou-Donia et al. 2002) (Table 6). Animal studies have documented signs of sarin-induced toxicity following treatment of male SD rats with single

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i.m. dose of 0.01, 0.1, 0.5, and 1 × LD_{50} (100 μg/kg) sarin (Khan et al. 2000). Fifteen hours after a treatment with only an i.m. 1 × LD_{50} dose of sarin caused a significant inhibition in brain AChE activity, and 20 h after treatment resulted in a significant increase in cortical and brainstem choline ACh transferase activity. Another treated group was terminated at 90 days and showed a significant decrease (~50% of control) in AChE activity. With exposure to a 1 × LD_{50} dose of sarin, AChE activity in the cortex and brainstem was decreased by ~29% and increased of 20% of control, respectively (Abdel-Rahman et al. 2002). AChE activity was not only reduced in the CNS with inhalation of sarin, but also was decreased AChE in the stomach, duodenum, jejunum, and ileum in guinea pigs four hours after inhalation of sarin; the AChE was still decreased in the duodenum and ileum after 24 h (Chanda et al. 2010). Effects were also seen in the liver, kidney, and spleen with inhaled sarin.

Effect of sarin on plasma BChE activity

Time-course studies of the effect of i.m. injections of 0.01–1 × LD_{50} (50 μg/kg) sarin on plasma BChE showed that only 1 × LD_{50} dose produced significant inhibition of plasma BChE activity from one hour to 20 h with peak inhibition at three hours, resulting in 40% of control. The enzymatic activity began to recover at 20 h to 50% of controls and was back to normal 15 days after dosing (Jones et al. 2000; Khan et al. 2000; Abou-Donia et al. 2002; Abdel-Rahman et al. 2002).

Effect of sarin on m2Muscarinic AChR (m2AChRs) ligand binding

A differential response in m2AChRs ligand binding in the cortex and brainstem was observed (Abdel-Rahman et al. 2002). The cortex showed a decrease in m2AChRs receptor ligand binding; however, in brainstem it exhibited an increase (~45% of control) at 1 × LD_{50} dose. Cortex also showed a biphasic response in nAChRs ligand binding at 0.1 and 1 × LD_{50} doses. A decrease at 1 and 3 h and increases at 6, 15, and 20 h in nAChR and m2mAChR binding were observed following 1 × LD_{50} (50 μg/kg) dose (Jones et al. 2000).

Effect of sarin on nAChR-ligand binding

A dose effect showed at 15 h after a single i.m. dose of 0.01, 0.1, 0.5 × LD_{50} (50 μg/kg) significantly increased cortex nAChR-ligand binding that accounts for 180%, 125%, and 140% of control, respectively (Khan et al. 2000). Ninety days after dosing with only 1 × LD_{50} sarin there was a decrease in cortex nAChR ligand binding to 62% of control while 0.5 × LD_{50} significantly increased brainstem nAChR to 120% of control (Jones et al. 2000).

Effect of sarin on sensorimotor performance

Dose–response effects of a single i.m. injection of 0.5, 0.75, 0.9, and 1.0 × LD_{50} (50 μg/kg) sarin on sensorimotor performance of SD rats was carried out at seven and 15 days after dosing (Abdel-Rahman et al. 2002). Each treatment resulted in significant sensorimotor impairment compared to controls, as shown in each of the behavioral tests at each time point. All doses of sarin caused significant impairment in performing the behavioral tests compared to control. Furthermore, there was a dose × time interaction for the beam walker score, incline plane performance, and grip time, indicating poorer performance on these tasks on day 15 versus day seven for at least one dose.

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Effect of sarin on the integrity of the blood brain barrier (BBB)

The effect of a single i.m. injection of sarin at 0.01, 0.1, 0.5, and 1 × LD_{50} (50 μg/kg) doses on BBB permeability was evaluated 24 h after treatment (Abdel-Rahman et al. 2002). Alterations in the permeability of the BBB were monitored using the positively charged [3H] hexamethium iodide. Only treatment with 0.5 × LD_{50} significantly increased BBB permeability only in mid-brain and in brainstem (138–168% of control). Furthermore, 1 × LD_{50} caused significant increases in BBB permeability in all brain regions ranging from 144 to 183% of control. Immunostaining with endothelial barrier antigen (EBA) that shows BBB protein in brain capillaries and also in smaller vessels in brain parenchyma has demonstrated that significant reduction in EBA staining is an indicator of changes in the BBB (Jensen et al. 1992). Animals treated with low doses of sarin, i.e. 0.01, or 0.1 × LD_{50}, has no effect on EBA immunostaining compared to control. In contrast, 0.5 × LD_{50} sarin caused occasional decrease in EBA immunostaining various regions of the brain. Furthermore, a dramatic decrease in EBA immunoreactive elements was noted in all brain regions 2–24 h after sarin treatment.

Sarin-induced neuronal cell degeneration

Although all treated male SD rats developed a seizure following a single i.m. dose of 1 × LD_{50} sarin, only half of the treated animals died within 15 min and the other half survived. The survivors exhibited brain neuronal death and breached BBB (Abdel-Rahman et al. 2002). Exposure to high doses of sarin caused increased permeability of the BBB in rats (Abdel-Rahman et al. 2002). There was evidence that sarin-induced cell death was accompanied by a release of cytochrome c (Abu-Qare & Abou-Donia 2001) and increased urinary excretion of 3-nitrotyrosine (Abu-Qare & Abou-Donia 2001). The results suggested that neuronal regional brain cell death was caused by the production of reactive oxygen species induced by sarin and subsequent apoptotic cell death. In addition to this cholinergic action, exposure to high doses of sarin resulted in severe pathological brain lesions accompanied by an alteration in behavior and cognition, especially impairment of learning and memory (Petras 1981; Abdel-Rahman et al. 2002). Recent studies showed that low-level inhalation of sarin caused long-term memory impairment and alteration of physiological functions in rats (Kassa et al. 2001). Morphologically, most of degeneration induced by 1 × LD_{50} sarin was seen in motor and somato-motor areas of the cerebral cortex, the dorsal thalamus, dentate gyrus, the CA1 and CA3 subfields of the hippocampus, and the cerebellum (Abdel-Rahman et al. 2002). Other areas of the brain only exhibited occasional dying neurons in some animals. Degenerating neurons were present in both superficial (I–III) and deeper (III–IV) layers of the motor somatosensory cortex from animals treated with 1 × LD_{50} sarin. In superficial layers, the majority of degenerating neurons were of pyramidal type, with prominent eosinophilic apical dendrites. In deeper layers of the cortex, degenerating axons were layer pyramidal neurons, mostly in layer II. Only a few degenerating neurons were observed in animals treated with 0.5 × LD_{50}, and no changes were found in animals given 0.01 or 0.1 × LD_{50} sarin (Abdel-Rahman et al. 2002). These results were confirmed with greatly reduced MAP-2 staining of dendrites in both superficial and deeper layers of the cortex. Animals treated with 0.5 × LD_{50} showed a slight reduction in MAP-2 immunostaining, while those treated with 0.01 and 0.1 × LD_{50} were comparable to control.

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A single 1 × LD_{50} dose of sarin caused neuronal degeneration in the dentate gyrus and CA1 and CA3 subfields of the hippocampal formation. In the dentate gyrus, degenerating neurons were present in both ranule cell layer and the dentate hilus (Abdel-Rahman et al. 2002). MAP-2 immunostaining, however, was not changed in the dentate gyrus (O'Leary et al. 1961; Masson et al. 2011; Newmark 2007). In both CA1 and CA3 subfields of the hippocampus the degenerating neurons were conspicuous in the stratum pyramidal (Abdel-Rahman et al. 2002). MAP-2 immunostaining showed greatly decreased expression of MAP-2 positive dendrites that also appeared fragmented and thinner in CA1 and CA3 sub fields of the hippocampus. No changes were found in sections for animals treated with 0.01 m and 0.1 × LD_{50} sarin (Abdel-Rahman et al. 2002). Purkinje cell damage was the most conspicuous alteration in the cerebellum in animals treated with 0.5 and 1 × LD_{50} sarin. Quantitative analysis showed a significant reduction in the number of Purkinje cells in these animals (Abdel-Rahman et al. 2002; Sawyer et al. 2012).

Treatment of sarin-induced poisoning

The current treatment for sarin exposure-induced neurotoxicity involves a combination of patient's management, antidotal treatment, and anticonvulsants, which are sometimes combined with pre-exposure prophylaxis (Newmark 2007). First, the patient should undergo airing airway by removal of secretions, administration of oxygen, and initiation of artificial respiration gastric lavage, if sarin is ingested, and decontamination of the skin. Victims of OP poisoning need to be decontaminated before entering a treatment center. Contaminated clothes should be removed and the victim should be washed with soap and water in order to reduce the risk of exposing more people to OP (Tokuda et al. 2006). Secondary exposure is also a major problem in treating sarin poisoning because first responders often do not have the right equipment to protect themselves from the damaging powers of the toxicants. Heavy butyl rubber gloves, boots, and respirators with self-contained breathing should be worn to help prevent exposure. Second, antidote treatment should be started as soon as possible.

Muscarinic receptors antagonist (atropine)

Generally, two antidotes are used, an anticholinergic and an oxime, which work synergistically. The most common anticholinergic is atropine, which is administered by an auto-injector in military or civilian mass causality scenarios. For individual patients with sarin poisoning, the usual administration is intravenous (IV) injection or intraosseous (IO), the process of injecting directly into the marrow of a bone. Atropine works as a competitive antagonist against the excess ACh by binding to the muscarinic receptors in the PNS and CNS. Atropine, however, cannot limit overstimulation of the nicotinic receptors nor can it reactivate the phosphorylated AChE. In fact, atropine provides purely symptomatic relief (Leikin et al. 2002). Furthermore, this medication induces its own anticholinergic toxicity if given in excess or in the absence of a true cholinergic crisis. These anticholinergic effects include blurred vision, dilated pupils, dry mouth, inability to sweat, tachycardia, and urinary retention.

Hydrolysis of phosphorylated AChE with 2-paralidoxime (2-PAM)

The second antidotal treatment, an oxime, acts as an AChE reactivator by hydrolyzing the phosphorylated enzyme and separating the nerve agent phosphate from the AChE active site

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(Kassa 2002; Figure 4). While oxime reactivation is effective at both the nicotinic and muscarinic receptors, the treatment is unable to reactivate the aged AChE-sarin complex or cross the BBB in high quantities. Therefore, the oxime must be administered before dealkylation has occurred. Like atropine, the oxime is also given via an IM auto-injector in military or civilian mass causality cases (McDonough 2002). 2-PAM is the oxime most commonly used in the United States. Its application is not expected to cause side effects when given at the doses necessary to reactivate AChE following nerve agent exposure (Leikin et al. 2002). 2-PAM, however, has been shown to cause dizziness, blurred vision and hypertension when given to healthy adults (Abou-Donia 1981). There is a wide range of oximes available worldwide in addition to 2-PAM.

The efficacy of a particular oxime is highly dependent on the identity of the nerve agent used as well as the animal species in which the study is performed (Dawson 1994; Kassa 2002; Kuca et al. 2006a, 2006b). An oxime has yet to be identified that is effective against all possible OPs. Studies have shown that the most efficacious AChE reactivators are the quaternary pyridinium aldoximes (Kuca et al. 2005). There are three main classes of oximes commonly used to treat nerve agent exposure: monoquaternary pralidoxime, diquaternary obidoxime, and HI-6. Of these, HI-6 appears to be the most potent reactivator of human AChE inhibited by sarin based on *in vivo* and *in vitro* studies (Kuca and Cabal 2005; Kuca et al. 2005; Worek et al. 2007). HI-6 has also been shown to reduce GABA inhibitory activity and pre-synaptic secretion of ACh (Shrot et al. 2009). Recent studies have shown that bis-pyridinium oximes connected by xylene linkers, such as N,N′-p-xylene-bis-(2,2′-hydroxyiminomethyl)pyridinium dibromide, are more effective at reactivating human AChE than currently available oximes (Acharya et al. 2010). The following treatment is recommended for an adult person following exposure to sarin (modified from Cannard 2006):

- Mild/Moderate symptoms: localized sweating, muscle fasciculation, nausea, weakness, and dyspnea.
  - Atropine 2–4 mg/kg i.m. (repeat every 5–10 min as needed).
  - 2-PAM chloride (15 mg/kg i.v. slowly, 2000 mg/h max).
- Severe symptoms: unconsciousness, convulsions, apnea, and flaccid paralysis.
  - Atropine 6 mg/kg i.m.
  - 2-PAM chloride 15 mg/kg i.v. slowly.

Other AChE reactivators

In attempts to identifying a universally applicable AChE reactivator, current research focus is on the synthesis of novel oximes. The efficacy of a particular oxime is often quantified as the protective ratio: the ratio of the LD_{50} of the nerve agent in the animal receiving treatment to that of the animal not receiving treatment (Dawson 1994). There are five structural characteristics that can affect the efficacy of an oxime: (1) presence of a quaternary nitrogen, (2) the length and rigidity of the chain connecting the pyridinium rings, if there are more than one, (3) the presence of an oxime, (4) the position of the oxime, and (5) the number of oximes (Kuca et al. 2006a, 2006b). In addition, the efficacy also appears to be correlated

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with the affinity of the oxime for the phosphorylated AChE (Kuca et al. 2005). There have also been studies on the use of multiple oximes in conjunction with one another to achieve more effective reactivation of AChE (Kassa et al. 2011). On the other hand, the study was unable to demonstrate improved outcomes when combinatorial therapies were used in comparison to HI-6 alone. Studies on the use of the tertiary oxime, monoisonitrosoactetone (MINA) have shown promising results in reactivating AChE in the brain (Skovira et al. 2010; Shrot et al. 2009). MINA has the ability to penetrate BBB, which is something that commonly used quaternary oximes struggle to do; therefore, it offers the potential for another component in the AChE reactivation approach. However, this line of therapeutic intervention is limited by re-inhibition of reactivated AChE by unreacted OPs that remain circulating in the blood following the exposure and by the inability of many of these drugs to cross the BBB leaving the CNS at serious risk.

Shielding of AChE by pyridostigmine bromide (PB)

PB, a quaternary dimethyl carbamate, is a reversible inhibitor of AChE that is used primarily for treatment of myasthenia gravis. This drug is fairly safe with a therapeutic dose of 200–1400 mg/day. PB is approved by the FDA as a prophylaxis against soman toxicity. During the 1990–1991 Gulf War, U.S. Service personnel used PB as a prophylaxis against possible attack with sarin at a dose of 30 mg with three daily doses every eight hours (McDonough 2002; Newmark 2007; Figure 5). PB acts by shielding AChE in the PNS by reversibly inhibiting 30–40% of the enzyme activity and protecting it from permanent inhibition by the nerve gas, sarin. Enzyme activity is restored following spontaneous de-carbamyalation of AChE resulting in a free enzyme and near-normal neuromuscular and autonomic functions (Blick et al. 1991).

The reversible inhibition protects a fraction of the soldiers' AChE from irreversible inhibition from the nerve agent, allowing it to be recovered later during the course of treatment. The target dose of PB inhibits 20–40% of an adult's available AChE (McDonough 2002). As the drug is itself an AChE inhibitor, it causes side effects such as exacerbation of asthma, intestinal hypermotility, miosis, salivation, and sweating. Studies, moreover, suggested that combinatorial exposure to PB and sarin can have long-term adverse effects and risks bromism as well (Abu-Qare & Abou-Donia 2001). These studies have shown that rats exposed to both sarin and PB have increased levels of 3-nitrotyrosine and 8-hydroxy-2′-deoxyguanoside (8-OhdG), markers of oxidative stress, in comparison to control rats. Some studies also used noncompetitive nicotinic antagonists. Combined exposure to PB, DEET, and permethrin caused sensorimotor deficit and alterations in brain AChE in rats (Abou-Donia et al. 2004). Turner et al. (2011) used the compound 1,1-(propane-1,3-diyl)bis(4-tert-butylpyridinium). Such compound was able to reverse the neuromuscular paralysis after nerve agent poisoning *in vitro* and to protect guinea pigs against poisoning by nerve agents when used with a muscarinic antagonist.

Bio-scavengers

Currently, investigations are under way to develop more effective treatments for OP poisoning, including nerve agents. A possible alternative choice to oximes are called "bioscavengers" (molecules that can bind and metabolize OPs in blood), e.g. BChE and

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human paraoxonase-1 (hPON-1) (Ashani et al. 1991; Nachon et al. 2013). These bioscavengers fall into two distinct categories: stoichiometric bioscavengers and catalytic bioscavengers. Stoichiometric bioscavengers bind to OPs in a mole-to-mole ratio, while catalytic bioscavengers degrade OPs with turnover and, therefore, can be used in smaller quantities (Nachon et al. 2013).

Stoichiometric bioscavengers (butyryl cholinesterase)

Human blood concentration of BChE (EC 3.1.1.8) is 2 mg/l. Although BChE has no known function, it functions as the first line of defense against poisoning with OP compounds. It acts as a bioscavenger, like a sponge to absorb and degrade OP compounds (e.g. nerve agents and insecticides, Radi et al., 2013; Abou-Donia et al., 2016). Although BChE represents the most promising stoichiometric bioscavenger for protecting against acute cardiac and neurological toxicity from OPs, it is unsuitable for clinical use due to the large quantities that are needed to treat a single patient (Saxena et al. 2008). The disadvantage of using a stoichiometric scavenger is a single molecule of OP binds to a single molecule of BChE molecule with a large molecular weight. Recent studies, however, suggested that the G117H mutation of BChE might work to reinforce its catalytic function against sarin. This observation was deduced from the mutant residue that skews the oxyanion hole and makes the phosphorus more open to the nucleophilic water molecule which improves the rate-limiting step in the catalytic activity of human BChE. Nevertheless, it is still inapplicable clinically (Yao et al. 2012). Another step in this field is the addition of oximes to these catalytic agents to improve their turnover rates. Most oxime reactivators of OP–AChE conjugates, however, are found to be less efficient in recovering OP–BChE activity (Sit et al. 2011). Radi et al. (2013) have presented more promising results; they showed better oxime-assisted recovery of catalytic functions when combining purified human BChE with an oxime reactivator with a distinctive structural scaffold. Currently, recombinant human BChE (rBChE) is being developed under the trade name Protexia® as a pre- and post-exposure therapy for OP compound poisoning. A recent study showed that all guinea-pigs survived when exposed to lethal doses of survived of VX when treated with Protexia®, compared with no survival in controls with no protection (Mumford & Troyer 2011). Protexia® is a pegylated rBChE that is formed by conjugation of the rBChE with polyethylene glycol in order to decrease rBChE immunogenicity, increase rBChE stability, and increase circulating serum of rBChE.

Catalytic bioscavenger, paraoxonase (PON)

Paraoxonase (EC. 3.1.8.1) belongs to the class A-esterase that hydrolyzes OP esters, carbamates, and aromatic carboxylic acid esters (La Du et al. 1991). The advantage of the use of catalytic scavengers is that they hydrolyze many OP molecules per each injection. Although BChE is the most widely suggested bioscavenger for OP toxicity, hPON-1 has been advocated as a possible alternative with better results. Recently, Valiyaveettil et al. (2011) used recombinant PON-1 (rePON-1) expressed in Trichoplusia (cabbage looper) in larvae against sarin and soman toxicity using micro instillation inhalation exposure in guinea pigs. PON1, a catalytic scavenger, hydrolyzes many OPs including paraxoxon, diazoxon, and chlorpyrifos oxon as well as the nerve agents sarin, soman, VX, and VR. Animals were pretreated i.v. with catalytically active rePON-1 followed by treatment with $1.2 \times LC_{50}$ sarin

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or soman. The administration of five units of rePON-1 led to a mild increase in the blood activity of the AChE enzyme after 30 min. Furthermore, this dose was able to protect the animals, resulting in a significant increase in survival and decreased signs of OP toxicity. The recombinant human paraoxonase-1 (rHuPON1) that was produced from *E. coli* was capable of protecting PON-1 knockout mice (PON1-1-) from exposure to high doses of the OP compound diazoxon (Suzuki et al. 2010).

Sarin-induced seizure

Definition: Seizures are an abnormal, unregulated electrical discharge that occurs within the cortical gray matter of the brain and transiently interrupts normal brain function (Sellner & Trinka 2012). A seizure typically causes altered awareness, abnormal sensations, focal involuntary movements, or convulsions (widespread violent involuntary contraction of voluntary muscles). The acute toxicity of sarin resulting in neuronal cell death is strongly associated with OP-induced seizures (Lallement et al. 1993).

The most problematic outcome of non-paralytic sarin exposure is its induced seizure and consequent brain damage. Seizures provoked by cholinergic agents are considered to progress in two phases: an early "cholinergic" phase and a subsequent "glutamatergic" phase, with a transition in between (McDonough & Shih 1997). The cholinergic phase lasts from the time of exposure to ~5 min after onset of the seizure. This phase is dominated by high CNS cholinergic activity; the seizure can be terminated by administration of an M1 muscarinic receptor antagonist at this time. Nicotinic receptor antagonists, in contrast, lack efficacy. Studies that used c-fos expression as a marker of neuronal activation identified the pyriform cortex, including a deep region known as the pre-endopyriform nucleus or "area tempests," as a potential trigger site for nerve gas-evoked seizures (Myhrer et al. 2008). Lesion studies (Myhrer et al. 2008) and pharmacological studies (Myhrer et al. 2008) provided results consistent with this hypothesis. With time, the seizure propagates from cholinoceptive neurons in the pyriform cortex (and medial septum) to glutamatergic limbic and neocortical circuits. Nerve agent-evoked seizures are complex partial (limbic) seizures that generalize secondarily and result in tonic–clonic convulsions. Thus, when seizures progress to the glutamatergic phase after 30–45 min, a drug that depresses glutamate transmission in limbic and neocortical regions would seem to be the ideal anticonvulsant. No such drug, however, is currently available for this use, and those tested to date have proved inadequate (Shih et al. 2003). Another possible choice would be a drug that enhances GABA-mediated inhibition. Indeed, several drugs of this type, including benzodiazepines, barbiturates, and propofol, are effective anticonvulsants during all phases of nerve gas intoxication (McDonough & Shih 1997). Unfortunately, all such drugs lose efficacy as the seizures progress (Chen & Wasterlain 2006), probably because GABAA receptors are internalized (Naylor et al. 2005). At the same time, AMPA and NMDA glutamate receptors are up regulated. Thus, NMDA receptor antagonists remain effective anticonvulsants in animal models, although a relatively high dose may be required.

Recently, Shih et al. (2003) reported that control of seizures induced by nerve agents was critical for neuroprotection and survival. These studies raised the possibility that excitotoxicity due to the activation of NMDA receptors and GABA-nergic pathways, in

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addition to the primary target, the cholinergic pathway, are important in sarin-induced neurotoxicity. Studies by Chebabo et al. (1999) provide data showing that the release of GABA is inhibited in the hippocampus following sarin exposure. From these studies, it is apparent that exposure with sarin may cause regulation of these pathways and play an important role in acute seizure and delayed neuronal cell death in different brain regions.

Mechanisms of sarin-induced seizure

While OP-induced cholinergic toxicity initiates the seizure, it is insufficient to account for all of the subsequent effects. McDonough and Shih (1997) have extensively reviewed the mechanism of OP-induced seizures as a three phase model consisting of: (1) an initial cholinergic phase which begins immediately upon exposure and lasts until approximately five minutes after the onset of the seizure, (2) the transitional phase, which begins more than five minutes after seizure onset and lasts until approximately 40 min after seizure onset, and (3) the non-cholinergic phase which begins approximately 40 min after seizure onset.

Initial cholinergic phase—In this phase decreased AChE activity results in the accumulation of ACh at the cholinergic receptors, as discussed in acute toxicity. The excess ACh then initiates the epileptiform activity. There is still some controversy surrounding the location of seizure initiation due to differences in the model organism chosen for study. Studies that exposed non-human primates to soman suggested that seizure activity begins in the amygdala (Lipp 1968). Alternatively, studies that exposed rats to soman have shown that seizures begin in either the cortical or subcortical regions or several thalamic nuclei (McDonough & Shih 1993; Collombet 2011). Furthermore, McDonough & Shih (1995) proposed that damage to these areas often begins at the piriform cortex and that this site may serve as a trigger site for soman-induced seizures. For rats exposed to VX via microinjection, the seizure appeared to initiate from within the limbic forebrain (McDonough et al. 1993). Additional potential seizure initiation sites include the area tempestas, medial septum, and perirhinal cortex (Myhrer 2010). When studying the first phase of seizures in guinea pigs, O'Donnell et al. (2011) noticed elevations in ACh and glutamate (GLU) levels in the brains of sarin-exposed animals. They also noticed that ACh levels in the striatum increased following triggering of seizure activity by sarin, indicating that this ACh response is the result of seizure activity.

The transitional phase of seizure induction—This phase is characterized by the transition between seizure initiation to the continuation of seizure activity (McDonough & Shih 1997). This secondary phase results from the disruption of the secondary neurochemical changes. These secondary neurochemical changes involve catecholamines: dopamine (DA) and norepinephrine (NE); excitatory amino acids: notably GLU and aspartate (ASP); and inhibitory amino acids: GABA and taurine (TAU). During the transitional phase, there is a decrease of NE and an increase on DA utilization. The levels of the excitatory amino acids increase between 5 and 20 min for GLU and after 30 min for ASP. Among the inhibitory amino acids, the levels of GABA are increased from 20 min following seizure onset and remain elevated for hours. Although these changes can occur during the initial cholinergic phase, they become more dramatic during the transitional phase. This elevated GLU activates α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

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(AMPA) receptors first and then later N-methyl-D-aspartate factor (NMDA) receptors. The activation of the NMDA receptors in conjunction with the increase in intracellular Ca2+ helps to propagate and maintain the seizure (Collombet 2011).

The non-cholinergic phase—This phase begins approximately 40 min following seizure onset. During this time, there is a sustained high level of GLU, an increase in the levels of DA, GABA, and TAU, and a decrease in the levels of ASP.

The primary effect of OP-induced seizures is the formation of neuropathological lesions. Once again, it is the neuronal cell death from the neuropathological lesions that is associated with OPICN. In animals, these lesions were only observed if the seizures lasted for longer than 20 min. The average duration of seizures necessary to cause damage was 40 min. Three main hypotheses have been suggested to account for the formation of neuropathological lesions resulting from OP-induced seizures: The excitotoxic hypothesis attributes the neuronal damage to the sustained seizure activity. The second hypothesis attributes the damage to oxygen deprivation from anoxia, hypoxia, and ischemia. The excitotoxic hypothesis is more probable as neuropathological changes are not observed when the seizure activity is blocked by benzodiazepines. However, the neuronal damage resulting from either of these pathways would result most directly from sustained toxic levels of interneuronal free Ca2+. The third hypothesis suggests that the OPs mediate neurotoxicity directly. This hypothesis is unlikely based upon *in vitro* studies that show that soman does not cause neurotoxicity in cultured hippocampal neurons (Deshpande et al. 1995).

Status epilepticus

Seizures and convulsions may progress to status epilepticus leading to neurodegeneration in the limbic system, including the hippocampus, amygdala, and piriform cortex. The formation of these lesions is due to neuronal death within the affected areas of the brain. Within 24 h of seizures in rats, a significant proportion of neurons in the central nucleus of the amygdala and in the hippocampus CA3 and CA1 pyramidal cell layers were affected. Similar results were reported in the adult rat showing that seizure caused neuronal loss in hippocampus fields CA1, CA3, dentate gyrus, dentate granule cell layer, and the dentate hilus. Prolonged epileptiform activity of these seizures promotes the release of excitatory amino acids, such as glutamate and ASP that are neurotoxic and have the potential to destroy central neurons. This seizure can result in severe brain damage and mortality if no early intervention takes place. Seizure-induced neuronal lesion is time-dependent, resulting in increased damage as seizure continues (McDonough & Shih (1995).

The mechanism of cell death was proposed to result from excessive excitatory neurotransmitter release that activates NMDA receptors and voltage-activated calcium channels allowing calcium to enter the cell. High levels of intracellular calcium leads to generation of reactive oxygen species through activation of nitric oxide synthase (NOS) that together uncouples oxidative phosphorylation in mitochondria, and activates the following enzymes: lipases, proteases, endonucleases, and other catabolic enzymes. These biochemical changes adversely affect cell function. However, these lesions often do not form immediately after exposure. Following the seizures, the degenerating neurons persist for two

weeks to two months possibly due to the cytokines, neurotrophic factors, and growth factors created in the inflammatory process that are known to have protective effects (Collombet 2011). Up to three months after the initial exposure and seizures, the degenerating neurons will continue to die possibly via apoptosis (Abdel-Rahman et al. 2002).

Excitotoxic levels of glutamate are thought to be involved in the dendritic and synaptic damage following acute OP exposure, leading to memory impairment. Over-stimulation of glutamate receptors results in the deterioration of synapses and cells in the hippocampus, a brain region involved in memory and cognition. Also, NMDA antagonists are used (Lallement et al. 1993). An NMDA receptor antagonist, such as MK-801, effectively blocks or terminates seizures.

Other cholinergic agents, including pilocarpine and the nerve gas agent sarin, evoke limbic motor seizures when administered to adult rats. No such studies on chlorpyrifos have been reported in the literature, but it is highly probable that chlorpyrifos-induced seizures in adult rats would be of the limbic motor type. Immaturity of limbic circuitry explains the absence of typical limbic motor seizures in these 16-day old rats. Limbic system conversant, such as kainic acid, are known to produce seizures in immature rats that differ from those expressed in adults.

Treatment of seizures

The GABA-nergic agonist diazepam is commonly used as an anticonvulsant drug. It acts by increasing the chloride current induced by GABA receptor activation that results in potentiating the effect of GABA throughout the nervous system. Recent studies indicated that protection with diazepam against brain damage is incomplete and is in agreement with the finding that seizures recurred after diazepam treatment following an initial period of seizure control (McDonough & Shih 1993).

The benzodiazepines are the only class of anticonvulsant treatments that are an effective treatment against OP-induced seizures (Newmark 2007). It has been proposed that the unique efficacy of the benzodiazepines is due to the distribution of cholinergic receptors within the CNS. Diazepam, however, also impairs attention and cognitive and psychomotor performance, specifically in critical flicker fusion threshold, decision-making, learning, and memory (McDonough 2002). Other seizure treatments look to target the specific sites of seizure initiation but, given that these regions vary by the type of OP administered and that each region responds to different pharmaceuticals, there is not a single treatment option that can offer complete OP protection (Myhrer 2010).

Recently, procyclidine has also shown promising results for its use as an anticonvulsant and neuroprotective agent when administered within 10 min of exposure in rats. These results were even better when the procyclidine was combined with Levetiracetam and HI-6 at terminating seizure activity and keeping the patient alive (Myhrer & Aas 2014).

Finally, efforts to regenerate the dead neurons include neuronal stem cells and cytokine therapy with fibroblast growth factor (FGF-2), epidermal growth factor (EGF), and brain-

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derived neurotrophic factor (BDNF) or nerve growth factor (NGF) to stimulate neuronal regeneration with appropriate connections (Collombet 2011).

Organophosphorus ester-induced delayed neurotoxicity; (OPDIN)

Organophosphorus esters are also associated with long-term effects known as OPIDN, which is a neurodegenerative disorder characterized by a delayed onset of prolonged ataxia and upper motor neuron spasticity as a result of a single or repeated exposure to OPs (Abou-Donia 1981). OPIDN is characterized by central-peripheral distal axonopathy following acute exposure at toxic dose levels or multiple low levels. The clinical picture for OPIDN is characterized by central-peripheral axonopathy that begins several days after exposure and is manifested initially by mild sensory disturbances, ataxia, weakness, muscle fatigue and twitching, reduced tendon reflexes, and tenderness to palpitation. In severe cases, the weakness may eventually progress to ataxia and paralysis.

OPIDN was first noted to develop in humans after consumption of TOCP-contaminated alcoholic beverage (Smith et al. 1930). Since then thousands of cases for OPIDN have been reported mostly following exposure to OP pesticides (Abou-Donia 1981). Furthermore, there is species sensitivity for OPIDN; hence, while rodents are not sensitive, cats, dogs, and chickens are sensitive to OPs, and the adult chicken has become the test animal required by the U.S. EPA to screen for OPIDN (Abou-Donia & Lapadula 1990).

Symptoms of OPIDN

The course of neurological deficits of OPIDN in human patients is divided into the following distinct phases (Smith et al. 1930; Abou-Donia 1981; Abou-Donia & Lapadula 1990).

Symptoms of OPIDN

The course of neurological deficits of OPIDN in human patients is divided into the following distinct phases (Smith et al. 1930; Abou-Donia 1981; Abou-Donia & Lapadula 1990).

- **Delay Period** of 1–4 weeks after exposure, but usually two weeks after a single exposure.
- **Progressive Phase** (lasts 1–6 months): early peripheral neuropathy characterized by numbness in fingers and hands, burning, tightness, and/or stinging calves of legs, and less often in the ankles and feet; bilateral dragging of the toes on the floor (foot-drop); bilateral and symmetrical flaccid paralysis, 2–4 weeks after exposure.
- **Stationary Phase** (lasts 3–12 months): sensory symptoms disappear, paralysis becomes stationary, bilateral paraplegia or quadriplegia persists.
- **Improvement Phase** (6–18 months after onset on neurologic deficits): improvement in the use of hands and arms and movement of feet and toes; in the severest cases, although hands show great improvement, complete paralysis remains below the knee.

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- **Long-Lasting Effect** (lasts 18–24 months): with time, the peripheral neuropathy is diminished and the long-lasting central lesions become unmasked and characterized as spasticity (excessive muscle tone or rigidity) and exaggerated knee jerk.

Prognosis

Functional improvement results from regeneration of PNS as CNS does not regenerate. It is possible, however, that acute reversible changes in the spinal cord, such as edema, might subside in time resulting in clinical improvement. Such improvement might also take place as other neurons with the same function take over the functions of the damaged neurons. Other neurons also may acquire the needed functions. In severe cases, permanent neurologic deficits occur and are often misdiagnosed as multiple sclerosis (Xintaras et al. 1978).

Mechanisms of OPIDN

Esterase as target for OPIDN

Early studies on the mechanisms of OPIDN focused on esterases. Inhibition of both AChE (Block and Hottinger 1943) and BChE (Earl & Thompson 1952) was proposed as the mode of action of OPIDN; however, subsequent studies eliminated both hypotheses (Aldridge 1954; Aldridge & Barnes 1966) that neither enzyme was the target for OPIDN. Johnson (1969) proposed the neurotoxicity target esterase (NTE) as the target for OPDN, and it was defined as an enzymatic activity preferentially inhibited by OP compounds capable of causing OPIDN. Many studies have investigated the mechanism of action of OPs that result in OPIDN. Some studies have proposed neuropathy target esterase (NTE) as the site of initiation of OPIDN, but other studies suggested that NTE may not be the only initiation site. For instance, Pope et al. (1993) utilized studies of the treatment of chickens with phenylmethylsulfonyl fluoride (PMSF) and diisopropylfluorophosphate (DFP) (Figure 1) to prove that NTE is not the molecular target of OPIDN. Although the NTE hypothesis generated intense interest in studying OPIDN and resulted in numerous publications, it did not advance the understanding of its mechanisms. NTE, however, is an excellent biomarker for OPIDN. For an OP ester to cause OPIDN, it must cause 70% inhibition of hen brain NTE following the administration of unprotected LD_{50} (Carrington & Abou-Donia 1985). A recent study has given convincing evidence against the NTE hypothesis showing that NTE-knockout mice are sensitive to the development of OPIDN, casting more doubt on the hypothesis that NTE is the target for OPIDN (Winrow et al. 2003; O'Callaghan 2003; Bus et al. 2003).

Kinases as targets for OPIDN

Protein kinases are able to amplify and distribute signals, since a single protein kinase is able to phosphorylate many different target proteins. Several protein kinases are turned on by second messengers. For example, calcium/calmodulin-dependent protein kinase II (CaM kinase II) is inactive until it is bound by the calcium–calmodulin complex that induces conformational changes and causes the enzyme to unfold an inhibitory domain from its active site. Further studies have identified a major role for this enzyme in the pathogenesis of OPIDN. An early event in OPIDN is an increased Ca2+ concentration in neuronal

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mitochondria of the hens' spinal cord (LoPachin et al 1988). This increase is followed by enhanced auto-phosphorylation (Patton et al. 1983, 1985, 1986), activity (Lapadula et al. 1991, 1992; Abou-Donia et al. 1993) and mRNA expression (Gupta et al. 1998) of CaM Kinase II. Activated CaM kinase II causes hyperphosphorylation of the cytoskeletal proteins: MAP-2; (Patton et al. 1983, 1985, 1986), tau (Gupta & Abou-Donia 1999), α- and β-tubulin (Gupta & Abou-Donia 1994; Suwita et al. 1986a, 1986b; Suwita & Abou-Donia 1990), neurofilament triplet proteins (Gupta & Abou-Donia 1995a; Gupta et al. 1999) and myelin basic protein (Abou-Donia 1995). Aberrant hyperphosphorylation leads to alterations in these proteins that are pathognomic of OPIDN. Elevated phosphorylation of MAP-2 reduces its ability to induce tubulin polymerization to form microtubules (Hoshi et al. 1988) and promotes disassembly of microtubules (Burns et al. 1984). Increased phosphorylation of tau reduces its ability to bind to microtubules and results in their destabilization with subsequent axonal degeneration (Gupta & Abou-Donia 1999). Enhanced phosphorylation of tubulin prevents its binding to MAP-2 or its polymerization to microtubules (Wandosell et al. 1986) and induces their aggregation into twisted polymers distinct from microtubules (DeLorenzo et al. 1982). Hence, chlorpyrifos induced tubulin phosphorylation that did not de-phosphorylate, but rather formed stable adduct (Jiang et al. 2010). Increased phosphorylation of neurofilaments prevented their assembly into filaments (Hisanaga & Hirokawa 1990); instead they form aggregates (Jensen et al. 1992; Gupta et al. 2000a) and exhibit slow axonal transport (Gupta et al. 1997). This abnormal axonal transport is also consistent with inhibition of caplain activity in hens' sciatic nerve (Gupta & Abou-Donia 1995a), leading to a decrease of neurofilament proteins in the spinal cord of hens treated with DFP (Gupta & Abou-Donia 1995b). This sequence of events leads to axonal degeneration and subsequent demyelination.

In the spinal cords of hens treated with DFP, there was an increase in medium (NF-M) and a decrease in low (NF-L) and high (NF-H) molecular weight neurofilaments (NFs) (Gupta et al. 1999). This imbalance in the stoichiometry of neurofilament proteins interferes with their interaction with microtubules and promotes neurofilament dissociation from microtubules, leading to their aggregation. Immunohistochemical studies in nervous system tissues from TOCP- and DFP-treated hens demonstrated aberrant aggregation of phosphorylated NF, tubulin and CaM kinase II (Damodaran et al. 2009, 2011).

Phosphorylation of these proteins interrupted their interaction, polymerization, and stabilization which lead to their degeneration. On the other hand, early studies identified transcription factors as critical phosphoproteins in signaling cascades. Immediate early genes control gene expression and therefore affect long-term cellular responses. The transcription of c-fos is elevated in OPIDN which may be the result of the activation of cAMP-response element-binding (CREB) which is phosphorylated by CaM kinase II (Damodaran et al. 2009). Subsequent to c-fos activation, CaM kinase II, neurofilaments (NFs), glial fibrillary acidic protein (GFAP) and vimentin had altered gene expression (Damodaran & Abou-Donia 2000) and increased protein kinase A activity (PKA; Gupta & Abou-Donia 2001), c-fos mRNA (Gupta et al. 2000b) and c-Jun mRNA (Damodaran & Abou-Donia 2000) in the brain and spinal cord of hens treated with TOCP or O,O-diisopropyl phosphorofluoridate (DFP).

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A toxicogenomic study conducted by Damodaran et al. (2006a, 2006b) also supports the hyperphosphorylation mechanism mentioned above. The study shows increased protein and mRNA expression of CaM kinase II in regions of the nervous system affected by OPIDN (Damodaran et al. 2006a, 2006b). Salama et al. (2014) proposes the possible role of mitochondrial complex I (CI) inhibition in the delayed effects of neurotoxicity. The study used hens treated with chlorpyrifos (CPF) and demonstrated signs of delayed neurotoxicity including NTE inhibition, CI inhibition and decreased ATP levels. Also, Jiang et al. (2014) demonstrated that disruption of glutamate-glutamine homeostasis in hens treated with TOCP might also contribute to OPIDN. Finally, a study in China by Zou et al. (2012) concludes that TOCP exposure causes changes in the autophagy-related proteins Atg1, Atg5, and Becln1 and these changes may contribute to OPIDN.

Incidences of OPIDN

Sarin-induced OPIDN in humans

Although available data suggest that sarin can cause OPIDN only at heroic doses, a recent report suggested that some humans may be susceptible to developing OPIDN following lower levels of sarin exposure. Exposure in the sarin attack in the Tokyo subway had variable results with some exposures resulting in serious health consequences. There was a report of a 51-year old man who was exposed to sarin during the Tokyo subway incident and survived its acute cholinergic toxicity and then developed neurological deficits consistent with OPIDN. He died 15 months after the incident and the neuropathological alterations were consistent with the dying-back degeneration of the nervous system characteristic of OPIDN. Distal sensory axonopathy in some subjects was observed three years after sarin intoxication in Tokyo (Himuro et al. 1998). This condition may be characterized as sarin-induced OPIDN superimposed on OPICN. Dysesthesia of distal parts of extremities were observed within a week in 16 patients in the Tokyo subway incident who were treated for acute intoxication that disappeared within one month. This symptom and its time-course are consistent with OPIDN. A total of 140 victims had pupil diameter <2 mm and 53 had serum BChE level <100. Some severely affected patients showed persistent sensory polyneuropathy, which is a characteristic of early stages of OPIDN.

Sarin-induced OPIDN in animals

A review by Munro (1994) provided evidence that at supralethal doses, sarin can cause OPIDN in antidote-protected chickens (Gordon et al. 1983). The tested doses ranged from 30 to 60 times the chicken i.m. LD_{50} of 25 μg/kg in hens protected with injected antidote. Abou-Donia (1981) reported in a review that a supralethal dose of 1 mg/kg produced OPIDN in hens. Similarly, Crowell et al. (1989) reported no significant inhibition of NTE activity was produced 24 h after oral administration of up to 6.6 times the maximum tolerated dose (4.0 mg/kg in atropine-protected hens). Also, daily oral doses of up to 1/3 maximum tolerated dose (MTD) for 42 doses in atropine protected hens that failed to produce signs of OPIDN. Sarin failed to produce OPIDN in cats treated with a lethal dose of 1 mg/kg, s.c. (LD_{50}: 0.035) in atropine/physostigmine-protected animals or after multiple doses of 0.0035 mg/kg/day for 10 days (Goldstein et al. 1987). No cases of OPIDN were

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reported in 246 human volunteers who were exposed to sarin via variety of routes (Crowell et al. 1989).

A large dose of sarin (5 mg/kg; route not specified) produced OPIDN in hens which were characterized by paralysis and neuropathological lesions in the sciatic nerve and spinal cord (Lancaster 1960). Repeated small and large lethal subcutaneous doses of sarin failed to produce OPIDN (doses' size and route of exposure were not specified) (Austin & Davies 1954). Administration of 26–28 daily LD_{50} doses (25 μg/kg dose, i.m.) of sarin produced OPIDN in hens protected with atropine and pralidoxime mesylate (oxime 2-hydroxy-iminomethyl-n-methylpyridinium methyl methanosulfate; P25) (Davies & Holland 1972). Sarin-induced delayed neurotoxicity was not observed in mice, unsusceptible species, or cats, susceptible species, (Goldstein et al. 1987; Himuro et al. 1998). A recent study in adult hens indicated that prior exposure to other "chemicals," i.e. PB (2 mg/kg/day p.o.), DEET (10 mg/kg/day, dermal), and permethrin (100 mg/kg/day dermal) for 60 days increased sarin's ability to cause OPIDN at 10 ug/kg i.m (0.1× LD50 dose (Abou-Donia et al. 1997). OPIDN was confirmed by the 60% inhibition of NTE; development of ataxia and paralysis in treated hens, and the presence of histopathological alterations consistent with OPIDN. The study also demonstrated that the combined treatment with sarin and chemicals caused severe inhibition of blood BChE and aryl esterase, both of which represent the first line of defense against sarin toxicity. Previous studies showed that PB and permethrin are primarily metabolized by both plasma BChE and aryl esterase as well as liver microsomal enzymes (Abu-Qare & Abou-Donia 2008) while DEET is mostly metabolized via cytochrome P450. Because sarin is metabolized by the same blood and liver enzymes, prior exposure to PB and permethrin increases sarin toxicity by impeding the bodies' abilities to detoxify and eliminate it. On the other hand, when sarin is applied alone, it is rapidly metabolized and eliminated and does not cause OPIDN. In contrast, prior exposure to the three chemical (sarin, PB, and permethrin) resulted in inhibition of plasma esterase with less enzymes available to detoxify sarin and, therefore, enhances its delivery into the brain. In effect, these chemicals "pump" more sarin into the CNS, leading to development of OPIDN at a sarin level that normally is below the threshold level to cause this effect. These results are consistent with the reported case of OPIDN in an individual who was exposed to sarin during the Tokyo attack.

Treatment of OPIDN

To date, there is no definitive treatment for OPIDN. There are, however, studies exploring possible ways to prevent and treat OPIDN in patients exposed to OPs. Such studies include efforts to restore calcium homeostasis that are involved in the activation of cytoskeletal proteins and the subsequent proposed chain of events leading to OPIDN. These studies also examined the effects of NTE inhibitors such as carbamates, thiocarbamates, sulfonyl fluorides, and phosphinate that can be administered prophylactically to prevent the development of OPIDN after OP exposure (Emerick et al. 2012).

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Organophosphate-induced chronic neurotoxicity

Definition: OPICN is an OP compounds-induced neurodegenerative disorder that is produced by a single large dose or by small doses leading to long-term structural, functional, physiological, neurological, neuropsychological, and neuropathological consequences and is largely characterized by chronic neurobehavioral alterations (Abou-Donia 2003; Loh et al. 2010; Chen et al. 2012). Individuals with OPICN develop a chronic neurotoxicity that is distinct from both the cholinergic action and OPIDN and persists for years after exposure. This action can occur following a single acute OP exposure at a toxic dose level or to small sub-acute exposures at sub-lethal dose levels, even in the absence of clinical signs of acute cholinergic toxicity. Within the brain, neuropathological lesions are seen in various regions which are characterized by neuronal cell death, resulting from early necrosis or delayed apoptosis and may be diagnosed as central and/or cerebral atrophy of the brain. The regions of the brain that exhibit neuronal degeneration include cerebral cortex, cerebellum, and the hippocampus, resulting in cognition and memory deficits as well as problems with emotions. Damage to these areas results in the following neurological deficits:

1. Cortex damage: results in dysfunctions in fine motor control movements, e.g. fingers, speech (laryngeal muscles). It also leads to muscle weakness and fatigue.

2. Hippocampus damage: causes cognition and memory deficits as well as emotional problems.

3. Cerebellum damage: results in change in gate (ataxia) and body imbalance, disorders in the coordination of hand and finger muscle, and body tremors.

The sequelae of OPICN have been recognized in 1990–1991 Gulf War veterans (White et al. 2001; Heaton et al. 2007) and the victims of the Japanese terrorist attacks (Yokoyama et al. 1998a, 1998b; Nishiwaki et al. 2001; Okumura et al. 2005). Victims of the terrorist attacks have continued to experience symptoms associated with OPICN for as many as seven years after the original attack (Miyaki et al. 2005).

OPICN following exposure to large dose of sarin

Two well-documented acute exposures to sarin were carried out by the “Aum Supreme Truth” cult (also known as “Aum Shinrikyo”) against civilians in Japan. At approximately 10:30 p.m. on 27 June 1994, 12 l of 70% sarin solution were released by evaporation using a heater and dispersed with a fan for 10 min in a subway in Matsumoto, Japan (Yanagisawa et al. 2006). The attack resulted in approximately 600 poisoned, eight dead, 56 admitted to hospitals, 208 taken to outpatient clinics, and 277 had symptoms of sarin poisoning, but did not request treatment. Three years after the Matsumoto attack in Japan, some patients complained of fatigue, shoulder stiffness, weakness, and blurred vision. Others complained of insomnia, bad dreams, husky voice, slight fever, and palpations. Some individuals who were acutely poisoned with OP compounds developed long-term impairment of their neurobehavioral performance (Colosio et al. 2003).

The second attack targeted civilians in a train in the Tokyo subway at 8:00 a.m. on 20 March 1995 (Murata 1997). The acute toxicity of sarin resulted in 12 deaths out of the 5500 civilians and rescue workers who developed immediate symptoms of sarin cholinergic

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toxicity. A total of 641 casualties suffered severe toxicity and required hospitalization on the day of the attack with an additional 349 being hospitalized within the week of the attack. Most of the exposed patients complained of mild symptoms and were discharged within a few hours. Symptoms of acute toxicity included miosis, dyspnea, nausea, headache, agitation, macular pain, blurred vision, muscle weakness, and fasciculation. Critical patients suffered cardiac or pulmonary arrest. Cholinergic toxicity was consistent with decreased BChE in 74% of the patients. A follow-up study six to eight months after the attack measured visual evoked potential (VEP) and brainstem auditory evoked potentials (BEAP) and documented that many of these victims developed signs and symptoms of OPICN (Murata et al. 1997), resulting from the long-term effects of sarin on the CNS, including insomnia, irritation, and nightmares. Neurophysiological studies of these victims showed a cognitive deficiency. The Romberg test revealed a significant effect on frequency of sway, suggesting sarin-induced effect on the vestibular-cerebellar system (Murata et al. 1997). Neural behavioral tests and post-traumatic stress syndrome (PTSD) checklist revealed that sarin victims scored lower in psychomotor performance and experienced fatigue. Over a year after exposure to sarin, symptoms such as persistent fatigue, headache, and ocular problems were further documented as OPICN (Okumura et al. 2005). Ohtani et al. (2004) documented the diagnosis of OPICN in some patients five to seven years after the sarin attack who continued to suffer from blurred vision, lack of focus, eye fatigue, reduced range of vision, headache, and fatigability. Seven years after the sarin attack, Miyaki et al. (2005) found declines in psychomotor performance and memory in a sarin dose-dependent manner.

OPICN following exposure to small doses of sarin

In addition to the individuals who were directly exposed to sarin in the Tokyo subway, other civilians and rescue workers were exposed to low level sarin vapors and subsequently developed OPICN. Two years and 10 months after this event in Tokyo, first respondents were divided into two groups of either high or low level exposure; they, subsequently, underwent testing for neurobehavioral performance (finger tapping) (Nishiwaki et al. 2001). The high exposure group showed a greater time between taps (measure of psychomotor performance) and exhibited maximal digit in the backward digit span test, indicative of neurobehavioral, memory performance, that also showed exposure level dependence, i.e. higher exposure, lower score. The low exposed group swayed more in the mediolateral direction with eyes opened in the stabilometric test. Out of a total of 58 persons who engaged in rescue work following the sarin attack, eight suffered subjective symptoms, one of whom was admitted to a hospital with symptoms of headache, nausea, and other symptoms. He had pinpoint pupils and conjunctional injection. Another patient was diagnosed with PTSD (Ohtani et al. 2004; Abe et al. 2006).

In other studies, in addition to acute cholinergic effects, healthy individuals exposed to a low dose of sarin have been reported to exhibit long-term neurological abnormalities (Sidell 1974; Burchfiel et al. 1976; Duffy et al. 1979; Burchfiel & Duffy 1982). Similarly, Moore (1998), Pearce et al. (1999), and Shih et al. (2006), observed a significant increase in beta2 amplitude in their cognitive behavior and electroencephalogram studies in the common marmoset following acute administration of low-dose sarin. A recent study based on the rescue personnel and police officers involved in the Tokyo subway sarin attack reported

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chronic decline in the memory functions of these personnel three years after the initial attack, suggesting even extremely low-level exposure to sarin could lead to long-term CNS effects (Nishiwaki et al. 2001). The victims exposed to sarin also scored lower on the memory function tests. While the memory function results at seven years were not statistically significant, these results confirmed the decrease in memory function related to sarin exposure that was observed at three years. Surveys of the victims of the two incidents in Japan were conducted five and 10 years after the attacks and noted that victims continued to suffer from asthenopenia, fatigue, headaches, blurry vision, and psychic conditions, including trouble concentrating, anxiety, and flashbacks (Yanagisawa et al. 2006). Between six to eight months after the exposure, the victims of the Tokyo attack demonstrated OPICN, as indicated by neurobehavioral and neurophysiological effects (Yokoyama et al. 1998a, 1998b). Specifically, the victims showed significantly higher scores on the General Health Questionnaire, indicating morbidity and on the Profile of Mood Stress tests, indicating fatigue. In addition, the victims scored significantly lower on the digit symbol neurobehavioral test, indicating deteriorating psychomotor function due to effect of sarin on the brain (Yokoyama 1998a, 1998b, 1998c, 2007).

Neuropathological characteristics

Neuropathological alterations are the hallmark of OPICN (Petras, 1981). They are different from neuropathological alterations of OPIDN or those induced by seizures and are consistent with neurological, neuropsychological, and neurobehavioral changes induced by OPICN. Yamasue et al. (2006) studied structural alterations in some victim's brains related to sarin exposure during the Tokyo attacks. The regional brain volumes were measured using diffusion tensor magnetic resonance imaging (DTI). The results indicated that although there was no change in the whole brain volume, compared to controls, the sarin victims exhibited reduced gray matter near the right insular cortex, temporal cortex, and left hippocampus. There also was reduced white matter near the left temporal stem. The pervasiveness of long-term symptoms was suggested to be a "by-product of structural changes in the brain" induced by sarin exposure (Yamasue et al. 2006).

Neurological changes

A recent study based on the rescue personnel and police officers involved in the Tokyo subway sarin attack reported chronic decline in the memory functions of these personnel three years after the initial attack, suggesting even extremely low-level exposure to sarin could lead to long-term CNS effects (Nishiwaki, et al. 2001). The symptoms of OPICN become apparent in the months following nerve agent exposure and can persist for years. Even seven years after the Tokyo sarin train attack, the victims still demonstrated significant psychomotor and memory impairments (Miyaki et al. 2005). There has been much interest in OPICN as a possible explanation for the various symptoms experienced by Gulf War veterans (White et al. 2001). The symptoms of OPICN become apparent in the months following nerve agent exposure and can persist for years. Even seven years after the Tokyo sarin train attack, the victims still demonstrated significant psychomotor and memory impairments (Miyaki et al. 2005). There has been much interest in OPICN as a possible explanation for the various symptoms experienced by Gulf War veterans (White et al. 2001). Nishiwaki et al. (2001) proposed that sarin exposure has a causal relationship with memory

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disturbance, although the mechanism has yet to be elucidated. Rats showed similar long-term memory deficits after repeated low dose exposure to sarin.

Neuropsychological

Some persons who exhibited reversible cholinergic toxicity at the time of the attack developed persistent neuropsychiatric deficits (Miyaki et al. 2005). First responders and even some exposed persons who exhibited no overt acute toxicity exhibited memory deficits 45 months after the attack. Thus, even low-level exposure to sarin may lead to long-term CNS dysfunction. Similar effects of low-level exposure to nerve agents were reported in American military personnel who had served in the 1990–1991 Gulf War. White et al. (2001) reported that personnel deployed in the war zone performed less well on cognitive tests in direct relation to their self-reported exposure to chemical warfare agents. Many of these individuals had been exposed to sarin when it was released into the atmosphere after destruction of the Iraqi chemical arsenal.

Neurobehavioral alterations

Long-term behavioral changes characterized by a decrease in activity, morbidity and gait have been observed in rats following sarin exposure (Kassa et al. 2001). Another study based on the rescue team staff members and police officers involved in the Tokyo subway sarin attack reported chronic decline in the memory functions of these personnel three years after the initial attack, suggesting even extremely low-level exposure to sarin could lead to long-term CNS effects (Nishiwaki et al. 2001). Some persons who exhibited reversible cholinergic toxicity at the time of the attack developed persistent neuropsychiatric deficits (Miyaki et al. 2005). First responders and even some exposed persons who exhibited no overt acute toxicity exhibited memory deficits 45 months after the attack. Thus, even low-level exposure to sarin may lead to long-term CNS dysfunction. Similar effects of low-level exposure to nerve agents were reported in American military personnel who had served in the 1990–1991 Gulf War. White et al. (2001) reported that personnel deployed in the war zone performed less well on cognitive tests in direct relation to their self-reported exposure to chemical warfare agents.

Prognosis

The symptoms of OPICN become apparent in the months following nerve agent exposure and can persist for years. Even years after the Tokyo sarin train attack, the victims still demonstrated significant psychomotor and memory impairments (Miyaki et al. 2005). There has been much interest in OPICN as a possible explanation for the various symptoms experienced by Gulf War veterans (White et al. 2001).

The signs and symptoms characteristic OPICN are similar to those that humans develop at old age. In other words, OPs, including sarin, seem to accelerate the process of aging. The clinical condition of patients with OPICN was further complicated by growing older when both signs of aging and OPICN overlap. Reports from sarin-exposed patients in Japan, who developed OPICN, indicate that in some cases there were some improvements. Because OPICN mostly results from damaged or dead CNS neurons that do not regenerate,

improvement results from other healthy neurons assuming the functions of damaged neurons.

Mechanisms of OPICN

OPICN can follow exposure to a single high dose of OPs, which causes acute toxicity or chronic exposure to subclinical doses of OPs (Abou-Donia 2003). Despite the established link between nerve agent exposure and OPICN, a distinct mechanism remains to be defined (Jamal & Julu 2002). Some possible mechanisms include: increased AChE gene expression, prolonged AChE inhibition, abnormal cerebral circulation, long term pre-synaptic disorder and variable synaptic safety factors, disturbed cellular protein turnover and transmembrane signaling, damaged proteins other than AChE and NTE, alteration of cytoskeletal proteins: microtubules, NFs, triplet proteins, inflammation (Banks & Lein 2012; RamaRao et al. 2011) and MAP; and CNS receptor deregulation: oxidative stress resulting in apoptosis and necrosis of the nerve cells (Jamal & Julu 2002; Abou-Donia 2003; Yokoyama 2007).

OPICN is associated with neuronal degradation, particularly in the CNS (Abou-Donia 2003). The neuropathological lesions are found in the cortex, hippocampal formation, and cerebellum, resulting from either apoptosis or necrosis depending on the dose, frequency and/or duration of exposure. Large doses that result in acute toxicity tend to cause necrosis, whereas chronic exposure to subacute doses tends to induce apoptosis (Hulet et al. 2002). Pilkington et al. (1999) demonstrated that the incidence of distal axon peripheral neuropathy was 40 times higher in OP exposed patients than in the general population. Long-term peripheral neuropathy was observed in victims of the Iran-Iraq conflict, which lasted from 1980 to 1988. This peripheral neuropathy seemed to correlate with OP chemical exposure; however, no CNS damage was reported (Holisaz et al. 2007). Another study assessed the gross neuroanatomical structures with quantitative MRI (Houston et al. 2001) in veterans of the Gulf War who were possibly exposed to sarin and cyclosarin (Heaton et al. 2007). This study showed that while there was no significant differences in the volumes of any inspected structures between the exposed and unexposed individuals, there was a significant decrease in the percentage of white matter and right and left lateral ventricle volume compared to the total volume.

Jamal and Julu (2002) extensively reviewed the link between acute and subacute nerve agent exposure and the symptoms characteristic of OPICN. Briefly, this review found that ~80% of the 19 studies focused on a positive link between low level OP ester exposure and the symptoms and signs associated with OPICN (Jamal & Julu 2002). Hulet et al. (2002) assessed the effect of subacute injections of sarin in guinea pigs. Using the highest doses of sarin that did not result in observable symptoms such as seizures or measurable toxicity as determined in a pilot study, it was shown that repeat injections over a two-week period prevented the habituation of the guinea pigs to handlers. This was in contrast with the control group, which did habituate to handlers. It must be noted, however, that in the full study the $0.5 \times LD_{50}$ dose did show signs of toxicity and resulted in the death of two of the nine experimental animals. The control guinea pigs and those treated with a $0.4 \times LD_{50}$ dose of sarin were significantly easier to handle by the end of the study than those that received a $0.5 \times LD_{50}$ dose. Neither the 0.4 nor $0.5 \times LD_{50}$ dose improved in approach and touch

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responses by the end of the study. There was a dose–response decrease in weight gain and a significant drop in the RBC AChE activity that persisted for up to 21 days into the recovery period for both doses of sarin as compared to the baseline. None of the surviving animals in the study, however, showed signs of neurodegeneration in the brain tissue obtained after sacrifice at the end of the experiment. In another study analyzing the dose–response effects of exposure to subacute doses of sarin in guinea pigs, behavioral performance was disrupted following repeat exposure to $0.4 \times LD_{50}$. This disruption included increased response times and decreased number of break points and response rates (Langston et al. 2005).

There is a well-established link between OP nerve agent exposure, neuropathological damage (Holisaz et al. 2007) and long-term behavioral differences (McDonough et al. 1986; Kadar et al. 1995; Jamal 1997). A recent study echoed the link between sarin exposure and neuropathological alterations (Grauer et al. 2008). Using rats exposed to $1 \times LD_{50}$, histological examination showed neuronal damage one week after exposure, concentrated in the hippocampus, the piriform cortex, and the thalamus. The level of damage correlated with the exhibition of convulsion activity at the time of exposure. Two additional measures of brain damage (prostaglandin [PGE2] levels indicative of inflammation and possible cholinergic cell death, and peripheral benzodiazepine receptor [PBR density] a marker of brain damage) were increased in the cortex and hippocampus of the convulsing sarin-exposed rats at four and six months following exposure. More recently, Oswal et al. (2013) suggested an alternative mechanism of the possible effects of low dose exposure to sarin on brain neurochemistry. In their study, levels of monoamines and their metabolites in different brain areas were analyzed after exposure of male C57BL/6 mice to a subclinical dose of sarin ($0.4 \times LD_{50}$). Despite the lack of neuropathological findings, a significant decrease in the DA turnover was observed in the frontal cerebral cortex (FC) of tested mice. Moreover, serotonin (5-HT) levels were transiently altered in the studied brain regions (increased in the FC and caudate nucleus and decreased in the amygdala).

Chao et al. (2014) used a 4T MRI to study the brains of 1990–1991 Gulf War veterans. This study demonstrated structural changes to the hippocampus, dentate gyrus, and parts of the Cornu Ammonis (CA), as well as a decrease in gray and white matter. Another study showed that cholinergic hyperactivity continues following an initial sub-lethal sarin exposure in rats, which can cause a depressed inflammatory system, lung hyper-reactivity, and a reduced threshold for epinephrine-induced arrhythmia that continued at least six months after exposure (Allon et al. 2011).

Organophosphates-induced endocrine disruption (OPIED)

An endocrine disruptor is defined by the U.S. Environmental Protection Agency (EPA) as "an exogenous agent that interferes with the synthesis, secretion, transport, binding or elimination of natural hormones in the body which are responsible for the maintenance or homeostasis, reproduction, development and/or behavior" (Rodier 1995; Grandjean & Lanrigan 2006; Louis & Stocker 2015).

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Endocrine toxicity of sarin

Research on the effects of sarin exposure on the endocrine system currently is sparse. Mach et al. (2008) found that exposure of mice to sarin and stress produced delayed endocrine effects as evidenced by the significantly enlarged adrenal glands and the significantly lowered concentrations of NE, epinephrine, DA, and other catecholamine. It is thought that combined exposure to sarin and stress can alter the neural pathways to the adrenal glands and inhibit AChE (Mach et al. 2008).

Low doses of sarin exposure were also found to significantly lower serum corticosterone levels for a long period of time (at least eight weeks after sarin exposure) and plasma adrenocorticotropin levels. The long term decrease in both serum corticosterone and plasma adrenocorticotropic levels indicates that hypothalamus–pituitary–adrenal axis suppression likely occurred due to sarin exposure, although the mechanism of action is not entirely clear (Peña-Philippides et al. 2007). Shewale et al. (2012) also demonstrated altered HPA responsiveness and reduced corticosterone levels following sarin exposure and postulates that the cause of this alteration was an autonomic imbalance.

Immunotoxicity of sarin

The immunosuppressive effects of sarin exposure include suppression of the Ab response; inhibition of Con A-induced T cell proliferation and anti-αβ-TCR-induced T cell proliferation; and inhibition of the anti-TCR-induced rise in Ca2+ concentration (Casale et al. 1982; Kalra et al. 2002; Kassa et al. 2003; Peña-Philippides et al. 2007). Peña-Philippides et al. (2007) found that subclinical doses of sarin were found to increase lung mRNA expression of IL-1β, IL-6 and TNF-α (proinflammatory cytokines) through an increase in nuclear content of NFκB, a transcription factor of the proinflammatory cytokines from F344 rats. Low doses of sarin, therefore, induce lung inflammation at the molecular level while suppressing T-cell immunity (Peña-Philippides et al. 2007).

Inhalation of low-level sarin at a symptomatic concentration was also found to significantly increase the production of N-oxides, which are required for peritoneal macrophages to kill microorganisms (Kassa et al. 2003). In addition, it was found that natural killer, YT, lymphokine-activated killer, and murine cytotoxic T lymphocyte activities were inhibited by several different OP pesticides. The mechanism of this inhibition is likely via the inhibition of serine proteases secreted by natural killer cells, lymphokine-activated killer cells, and cytotoxic T lymphocyte cells to induce apoptosis (Li et al. 2002). Yet another possible mechanism through which sarin acts on the immune system is through cholinergic stimulation. ACh may act directly upon components of the immune system, such as stimulation of antibody exposure to sheep erythrocytes in inbred mice (Casale et al. 1982).

Sarin suppressed T-cell responses and altered regional but not total brain AChE activity. Low-level inhalation of sarin to inbreed BALB/c mice showed that not only symptomatic but also an asymptomatic dose of sarin was able to alter the reaction of the immune system at one week after exposure (Kassa et al. 2003). While the number of CD3 cells in the lungs was slightly decreased, an increase in CD19 cells was observed, especially in the lungs and blood. The reduced proportion of T-lymphocytes is caused by the decay of CD4 positive T-

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cells. The production of N-oxides by peripheral macrophages was stimulated by symptomatic sarin concentration, whereas the natural killer cell activity was significantly higher following asymptotic sarin level. A study was carried out on the response of F344 rats to low levels of sarin that causes no overt clinical signs (Henderson et al. 2002). Inhalation levels were 0, 0.2, or 0.4 mg/m^3 of sarin for 1 h/day for 1, 5, or 10 days at room temperature and heat-stressed (32 °C) and observations were made one month after each of the exposures. Auto-radiographic studies showed that M1 mAChR site densities were unchanged after one day, but there was a decrease in M1 receptors in the olfactory tubercle, frontal cortex and hippocampus with or without heat and with heat stress. Sarin alone did not affect M3 mAChR. In combination with heat, however, there was an upregulation in number of binding sites in the frontal cortex, olfactory tubercle, anterior nucleus, and striatum immediately after exposure that persisted for 30 days. Sarin alone reduced AChE in the cerebral cortex, striatum, and olfactory bulb. Combined exposure to sarin and heat decreased AChE staining in the hippocampus that may be associated with memory loss and cognitive dysfunction.

Delayed behavioral and endocrine effects were studied following subcutaneous exposure to sarin ($3 \times 0.4\ LD_{50}$) combined with intermittent shaker stress in C57BL/6J mice (Mach et al. 2008). This combination caused delayed behavioral change manifested as excessive grooming and endocrine alterations in adrenal glands seven weeks after sarin exposure. In conclusion, these findings indicate that sarin in low doses is more dangerous when combined with shaker stress.

A recent study found no evidence of developmental toxicity in the CD rat or NZW rabbit following exposure to either Type I or Type II sarin during embryonic differentiation and major organogenesis, even at doses that produced maternal toxicity (LaBorde et al. 1996).

Reproductive and developmental toxicity of sarin

In 1982, the Surgeon General of the U.S. Army issued a warning against the possible teratogenic effects of sarin after the teratogenicity of another OP compound structurally similar to sarin was confirmed (LaBorde et al. 1996). LaBorde et al. (1996) found no evidence of developmental toxicity in the fetuses of pregnant rats treated on days 6–15 of gestation and pregnant rabbits treated on days 6–18 or exposed orally to both Type I and Type II sarin (Type I and II differ based on the stabilizer included [tributylamine and diisopropylcarbodiimide, respectively]) at various doses after gestation day 6 during "the period of important embryonic differentiation and major organogenesis." In fact, no significant alterations in normal fetal development, such as structural malformations or changes in fetal weight, were observed despite the observable maternal toxicity. Similarly, studies testing exposure to sarin vapor at various doses and for various durations in SD rats did not produce either developmental or reproductive toxicity (Denk 1975; Opresko et al. 2001). The results of studies assessing toxicity with inhalation exposure are especially relevant as this is also the most likely route of exposure in humans (Cannard 2006).

Due to the cholinergic toxicity of sarin, exposure during pregnancy could potentially cause developmental toxicity. In fact, long-term neurobehavioral effects have been associated with

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in utero exposure to VX, another OP nerve agent which also inhibits AChE activity (Munro 1994).

Sarin-induced alterations in gene expression

Expression of cholinergic system

Sarin does not only affect proteins already existing within the cell, but it can also cause alterations in gene expression which can alter the amounts of certain proteins leading to more profound effects. Damodaran et al. (2006a, 2006b) demonstrated that rats exposed to $0.5 \times LD_{50}$ sarin had altered mRNA levels for proteins that code for ion channels as well as cytoskeletal and adhesion molecules. These changes were shown to persist up to three months after the rats were initially exposed to sarin. Studies have also shown that sarin may exacerbate the abundance of ACh in addition to the inhibition of AChE. Choline acetyl transferase (ChAT) levels were elevated following sarin exposure. ChAT is an indicator for ACh biosynthesis as it participates in the last stage of the synthesis process. Therefore, in addition to inhibiting ACh breakdown by AChE at the neural synapse, sarin increases ChAT activity and subsequent synthesis of Ach, thereby, creating a twofold attack on the cholinergic system (Khan et al. 2000). The effects of sarin that altered gene expression may help to explain some of the long-term effects of sarin exposure.

Effect of sarin on mRNA for GFAP and vimentin

The expression profile of mRNA for GFAP, vimentin, and tubulin was studied in various brain regions of male SD rats. Male, SD rats were treated with a single i.m. dose of sarin (0.5 × LD50, 50 μg/kg) at 1 and 3 h and 1, 3, and 7 days. There was a significant induction of mRNA for GFAP and vimentin in cortex, brainstem, midbrain, cerebellum, and spinal cord. Both the GFAP and vimentin were induced at early time points in all the regions, except brainstem where moderate to high levels were observed at 1 and 3 days, respectively, following treatment with sarin. These data indicate that the development of long-term, sarin-induced neuropathological abnormalities may involve changes in the gene expression pattern of the components of astroglial cytoskeleton (Damodaran et al. 2002).

Effect of sarin on mRNA for tubulin

There was a significant induction of tubulin mRNA for tubulin in cortex, brainstem, midbrain, cerebellum, and spinal cord. The expression showed a spatio-temporal difference in each region, similar to the expression pattern of other cytoskeletal proteins such as GFAP and vimentin. These data indicated that the development of long-term, sarin-induced neuropathological abnormalities involved changes in the gene expression pattern of the components of astroglial cytoskeleton (Damodaran et al. 2002).

Sarin-induced expression of brain AChE mRNA

A single i.m. dose of $0.5 \times LD_{50}$ sarin administered to rats caused differential induction and persistence of AChE mRNA levels in different regions of the brains. One hour after dosing, sarin induction of AChE transcripts in various brain regions was in (% of control): brainstem, 126%, cortex, 149%; midbrain, 153%; and cerebellum, 234%. In brainstem,

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AChE expression level increased over time and then remained elevated after a decline at one day. In midbrain, AChE mRNA reached a peak level 2 h after administration, decreased to control level at day 1, then increased and remained elevated for day 3. In the cortex, transcript levels came down to control level by day 1. The increases in the cerebellum, which had the largest increases at 1 and 2 h after sarin administration, declined to control level by day 1. AChE gene expression is an important step in the process of the cholinergic neuronal systems for the maintenance of CNS homeostasis.

These results are consistent with previous finding that 15 days after a single dose of sarin, there was an increased activity in AChE (Abou-Donia et al. 2002), and CHAT (Khan et al. 2000) that followed their initial inhibition. In brainstem, AChE gene expression correlated with the highest inhibition of AChE and CHAT at an earlier time, its recovery to control levels at 24 h (Khan et al. 2000) as well as increased enzyme activity at a similar dose at 15 days (Abou-Donia et al. 2002).

In a similar way, immediate induction and persistence of inducted levels of AChE mRNA in midbrain can be correlated with an initial inhibition of AChE enzyme levels (Khan et al. 2000) followed by an increase of enzyme activity at 15 days. This modified level of transcription can also be explained by the combined effect of direct inhibition of the cholinergic pathway coupled with the activation of other apoptotic or necrotic pathways.

Factors affecting neurotoxicity of sarin

Combined exposure to sarin and PB

The effects of PB and sarin alone and in combination were investigated on sensorimotor behavior and the central cholinergic system of rats treated with a single oral dose of PB (13 mg/kg, gavage) and a single i.m. dose of sarin (80 μg/kg). Treatment with either PB or sarin alone resulted in significant sensorimotor impairments that continued to worsen with time. Cortical AChE activity was inhibited in the animals treated with each dose of sarin alone and in combination with PB. Cortex and brainstem m2mAChR ligand binding showed significant increases following co-exposure to PB and sarin at higher doses. Combined treatment with sarin and PB resulted in a significantly increased levels of urinary levels of 3-nitrotyrosine and 8-hydroxy-2′-deoxyguanosine at 48 h after the treatment and stayed elevated up to 96 h following the treatment (Abu-Qare & Abou-Donia 2002). Treatment with either PB or sarin alone did not cause an increased excretion of these biomarkers of oxidative stress in the urine (Abu-Qare & Abou-Donia 2001).

Heat stress

Henderson et al. (2002) and Chen et al. (2012) found that while heat stress reduced weight gain, sarin did not affect body weight. Combined exposure to sarin and heat stress resulted in lower breathing frequencies than when sarin exposure occurred at normal temperatures. Moreover, RBC AChE activity was reduced by 60% upon repeated higher subclinical dose exposure to sarin and heat stress. Although AChE staining was reduced in the cerebral cortex, striatum, olfactory bulb and areas of the forebrain and unchanged in the brainstem and cerebellum, brain AChE activity was not significantly reduced. Heat stress reduced

AChE staining in the hippocampus, which is an area vital for memory and learning. A significant reduction in M1 muscarinic receptors occurred in the olfactory tubercle under sarin only exposure, while exposure to sarin and heat stress resulted in a loss of M1 muscarinic receptors in the frontal cerebral cortex, the olfactory tubercle, the anterior olfactory nucleus, the striatum, the dentate gyrus, and the CA1 region of the hippocampus. M2 receptors were not affected and M3 receptors were upregulated only under simultaneous exposure to sarin and heat stress and were associated with delayed effects.

The effect of exposure of male SD rats to a single i.m. dose of $0.5 \times LD_{50}$ (50 μg/kg), heat stress (38 °C for 4 h), alone or in combination were studied (Shehata and Abou-Donia, unpublished results). Heat exposure significantly inhibited AChE activities in most brains and increased plasma BChE activity. On the other hand, sarin inhibited the activities of both brain AChE and plasma BChE activities. These results are consistent with heat-increased AChE in chick muscle cultures (Eichler et al. 1991). Heat inhibition of AChE might have resulted from heat-induced changes in the structure of the enzyme (Görne-Tschelonkow et al. 1993). Heat-enhanced activity of BChE might have been caused by alteration in its secondary structure. The active site of this enzyme is located at the bottom of a deep and narrow gorge, lined with eight aromatic residues (Harel et al. 1992). On the other hand, sarin significantly inhibited both brain AChE and plasma BChE activities consistent with previous finding (Khan et al. 2000).

Neither heat nor sarin alone had any effect on M2-MAChR ligand binding in all brain regions. On the other hand, combined heat and sarin exposure greatly increased M2-MAChR ligand binding in rat midbrain.

Permeability of the BBB was measured by determining radioactivity following oral administration of [3H]-PB. Heat increased BBB permeability in the cortex, midbrain, and brain stem. Sarin alone or in combination with heat increased BBB permeability in all areas of the brain. These results are in agreement with other studies (Black 1995; Greenwood & Johnson 1995). Previous studies have shown that AChE inhibitors increase BBB permeability (Abdel-Rahman et al. 2002).

Psychological stress

Mach et al. (2008) assessed delayed behavioral effects, blood BChE activity, adrenal weight, and catecholamine content in the mice exposed to sarin and stress. The results showed significant decline in blood BChE activity that was augmented by stress, a significant increase in adrenal weight and significant declines in the catecholamine concentrations of NE, epinephrine, and DA. Mice also displayed excessive grooming behavior three to four weeks after the combined exposure.

Pharmacogenetics

Pharmacogenetics investigate the mechanisms of individual variations in drug metabolism. Pharmacokinetic conditions are usually, detected following exposure to a xenobiotic, rather than disturbance of internal metabolism of an endogenous compound (La Du et al. 1991). Examples of this condition are esterases that act as the body’s first line of defense against OP compounds such as sarin. There are two main classes of esterases: aliesterases and aryl

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esterases. Both groups have several polymorphisms that determine their ability to metabolize and detoxify organophosphates and subsequently the toxicity of these chemicals.

Butyrylcholinesterase (BuChE)

This enzyme plays a major role in the protection of humans from the toxicity of sarin. Once inside the body, BChE acts as scavenger of OPs such as sarin and rapidly hydrolyzes it to less toxic metabolites (Abou-Donia et al., 2016). This enzyme has a variant called "atypical" BChE that has a diminished capacity to hydrolyzed substrates such as succinylcholine and sarin (Kalow & Davis 1958; Lockridge 1990).

Paraoxonase (PON1)

Paraoxonase (EC 3.1.8.1) an A-esterase catalyzes the hydrolysis of paraxon, diazoxon, chlorpyrifos oxon, and phenylacetate (Costa et al. 2013). Paraoxonase is synthesized in the liver and some of it is secreted into the plasma where it is associated with high density lipoprotein (HDL). It has two genetically determined polymorphic forms: The A type that has relatively lower paraoxonase activity, and the B type, with much higher paraoxonase activity. The function of this enzyme is the detoxification of organophosphate chemicals. There is also species selectivity regarding the expression of this enzyme. Birds have no detectable plasma paraoxonase activity, which explains their sensitivity to toxicity induced by these chemicals as indicated by their very low LD_{50} values for several organophosphates (Walker & Mackness 1987).

Overall mechanisms of sarin-induced neurotoxicity

Organophosphorus insecticides were the first group of insecticides to be "rationally designed as insecticides" in 1938 in Germany. Previously, insecticides were used because their insecticidal activity was discovered, and then their mode of action was later studied, mostly as an academic curiosity. Examples of these insecticides are pyrethroids, i.e. pyrethrum and chlorinated hydrocarbon insecticides such as DDT and its analogs. It is interesting that even before the development of Ops insecticides, the industrial chemical, tri-ortho-cresyl phosphate (TOCP) was discovered to cause "demyelinating disease" (Smith et al. 1930), that resulted in the paralysis of tens of thousands of Americans who consumed TOCP-adulterated ginger extract, an alcoholic beverage. This condition was latter designated OPIDN (Abou-Donia 1981). Although OP insecticides were designed to inhibit AChE, several reports indicated that exposure to high or low levels of these insecticides leads to "long-term" neurotoxicity characterized by neurobehavioral and neuropsychiatric disorders. More recently, this condition was characterized as a neurodegenerative disorder, known as OPIICN (Abou-Donia 2003). In this review we define the last, and least known action of Ops, as OPIED.

Figure 6 is a schematic presentation that summarizes of all of these actions attributed to Ops including saran as follows:

1. Cholinergic neurotoxicity results from acute exposure to Ops, leading to inhibition of AChE and accumulation of ACh at the muscarinic and nicotinic

AChRs leading to the development of the "cholinergic crises" that could progress to death.

2. Seizures may develop in some individuals following exposure to high levels of Ops or even following low-levels of these chemicals in children and sensitive individuals. This effect is accompanied by neurodegeneration and neuronal cell death.

3. OPIDN may develop following exposure to certain ops, including sarin resulting in neuronal degeneration and may lead to paralysis that could progress to death. This action seems to involve increased activity of CaMKII and hyper-phosphorylation of cytoskeletal proteins, leading to disruption of axonal transport and development of reactive oxygen species followed by neuronal cell death.

4. OPICN may also develop following exposure to high or low-levels of OPs, leading to the generation of ROS and apoptotic cell death.

5. OPIED may also develop following exposure to high or low levels of OPs, including the nerve agent sarin.

Conclusions

Sarin was named for its discovers in 1938, **S**chrader, **A**mbros, **R**itter, and Van der L**in**de. Its synthesis, use, and impact on human life can be described in one word: tragic. After it failed to qualify for use as an insecticide in 1938 because of its severe acute toxicity, it was dedicated for one purpose only that is "killing humans." Initially, sarin was used as a nerve agent and tested on innocent people in concentration camps during the holocaust in World War II (Karenberg, 2006). Since then, sarin has become the chemical weapon of choice for terrorists, both fanatics and governments. The international community must develop concrete plan to eliminate this chemical similar to the processes used for elimination of dangerous diseases such as small pox and polio. Following its unfortunate use in Japan, the Japanese scientists have done an exemplary job of documenting its short- and long-term actions following massive and small-level exposures. The results of their studies, have been very useful in defining sarin's action and in particular that of OPICN.

The review of numerous studies from a PubMed search of cholinergic and acute neurotoxicity, sarin and seizure, sarin and OPICN or sarin and chronic toxicity, sarin and endocrine, and sarin and OPIDN or sarin and delayed neurotoxicity from 1975 to 2015 (Table 6) is a schematic presentation of proposed mechanisms, symptomology, and treatment of various actions of sarin, i.e. acute toxicity, seizures, OPIDN, and OPICN after sarin exposure (Table 7 and Figure 6). These studies have also exposed the various shortcomings of the information surrounding OP poisoning. There is still much to be learned in this area of study, but this paper attempts to combine and summarize the information currently available in a concise manner.

There is sufficient evidence to suggest that sarin exposure has both short and long-term consequences and that these effects may be exacerbated by various other factors including

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heat and stress. There is also an indication that these conditions may explain the symptoms of the 1990–1991 Gulf War Syndrome (Research Advisory Committee on Gulf War Veterans' Illnesses, 2008). There is evidence that sarin-induced damage to the body occurs in a three-pronged, multi-faceted assault; further studies should focus on reducing this sarin-induced damage and obtain a greater understanding of the mechanism behind each of these stages of sarin toxicity. The ultimate goal would be the development of better methods for the treatment of patients' post-OP exposure to reduce acute, delayed, and chronic neurotoxicity.

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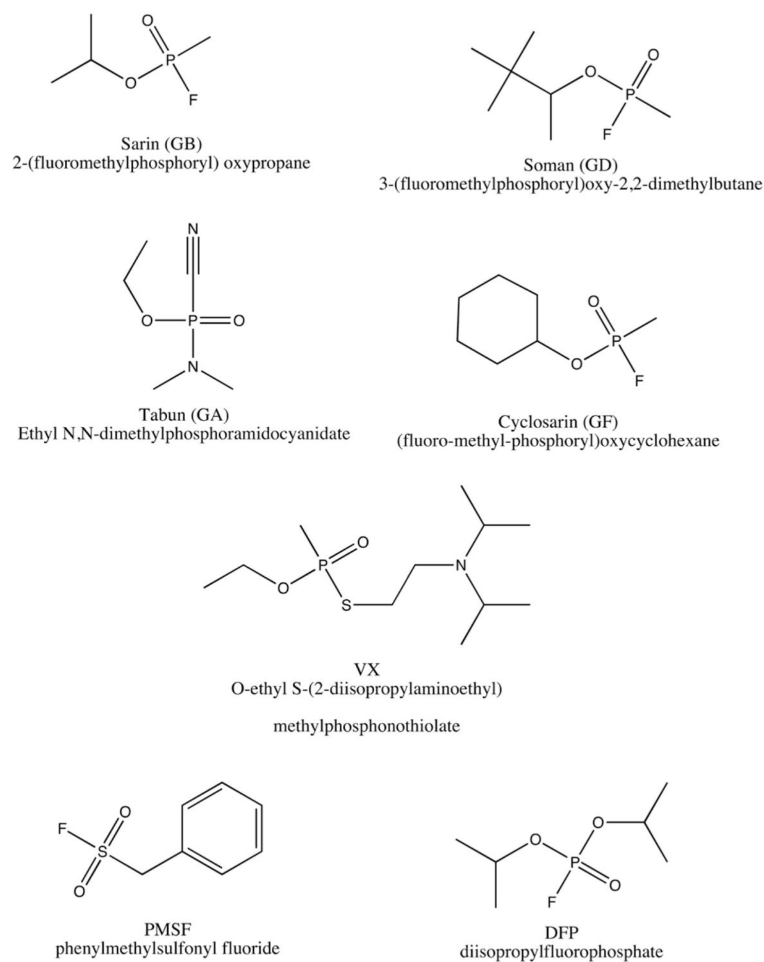

Figure 1.

Structure of the organophosphorus nerve agents molecules and related chemicals.

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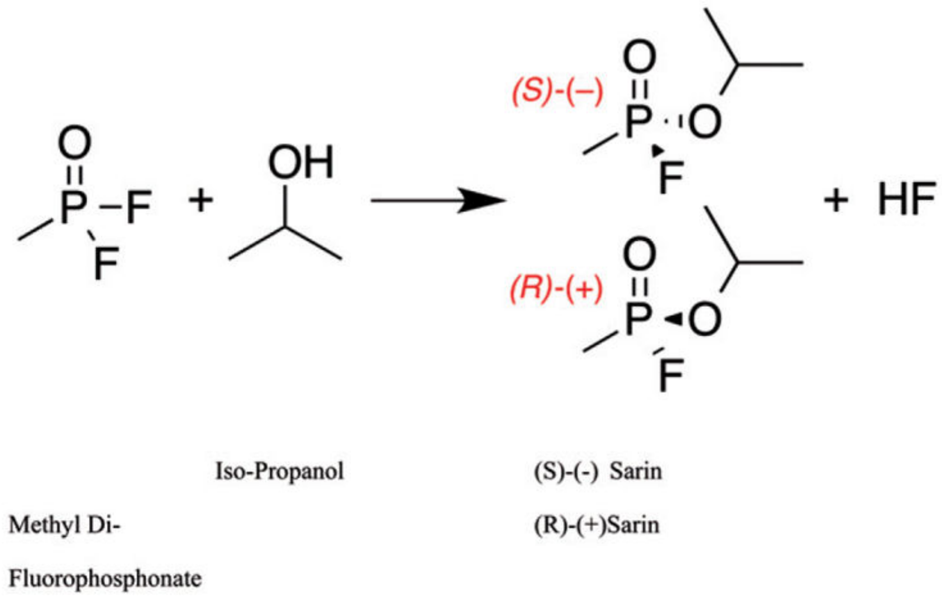

Figure 2.
Synthesis of sarin.

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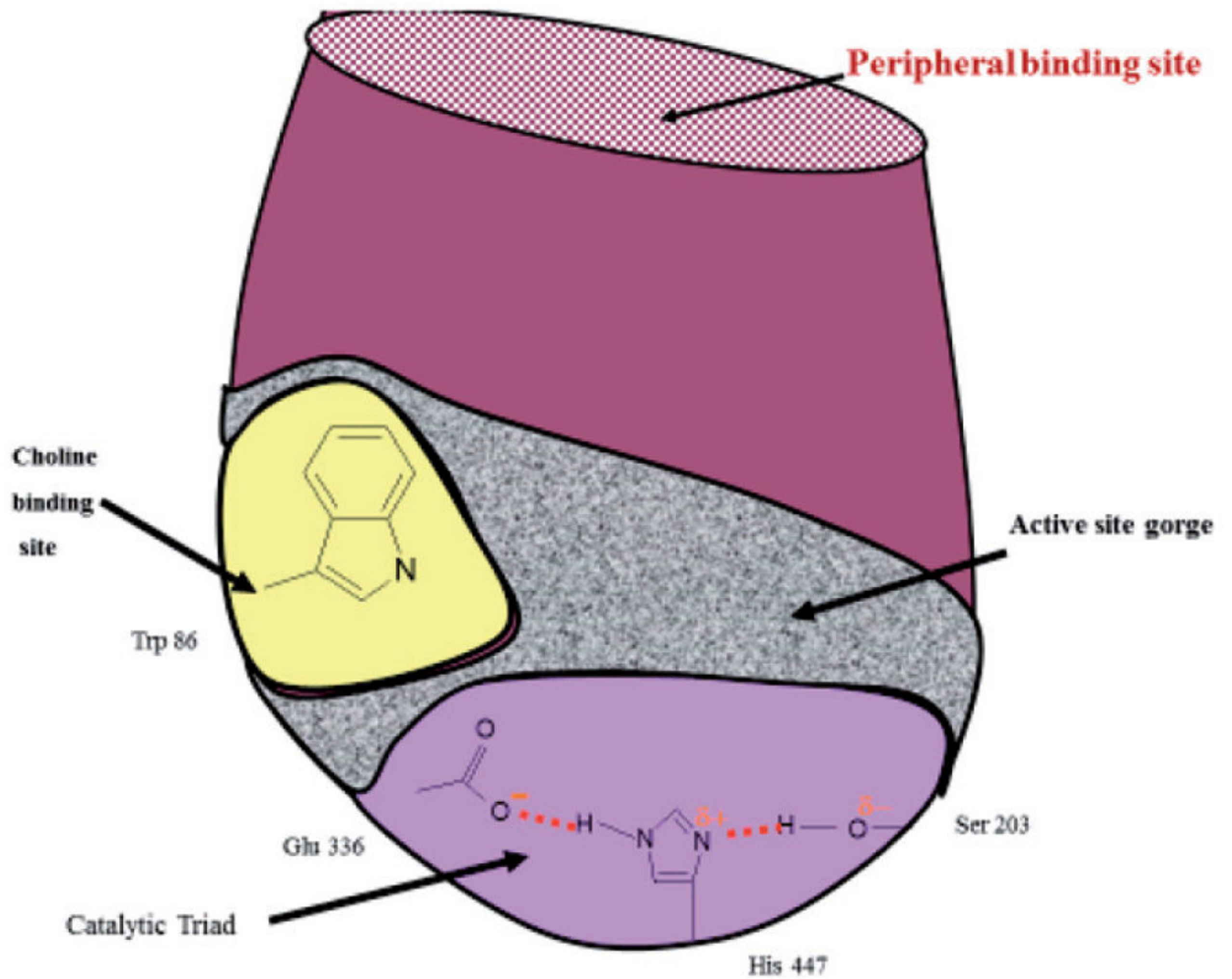

Figure 3.
Active center gorge of mammalian acetylcholinesterase including: active site gorge, Catalytic triad, a choline binding site and a peripheral binding site (Abou-Donia 2003).

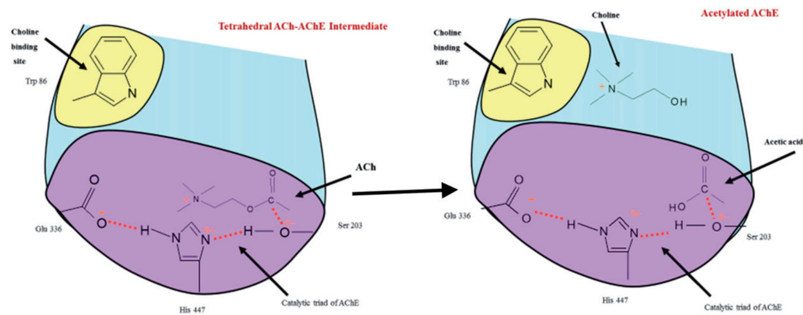

Figure 4.

Hydrolysis of acetylcholine (ACh) by acetylcholinesterase (AChE): A) AChE/ACh complex, B) acetylated AChE (Abou-Donia 2003).

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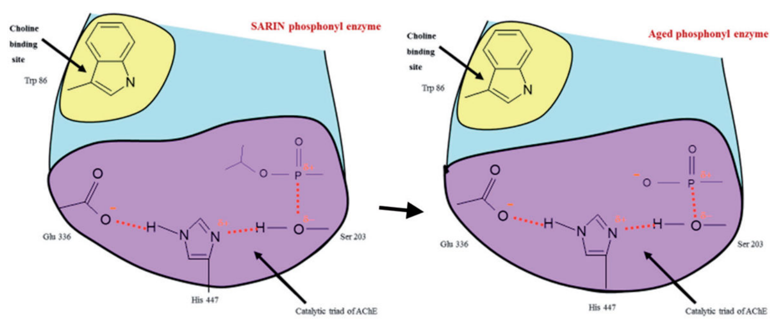

Figure 5.

Sarin inhibition of acetylcholinesterase (AChE). A) Isopropyl methylphosphonyl AChE: B) Aged Phosphonyl AChE.

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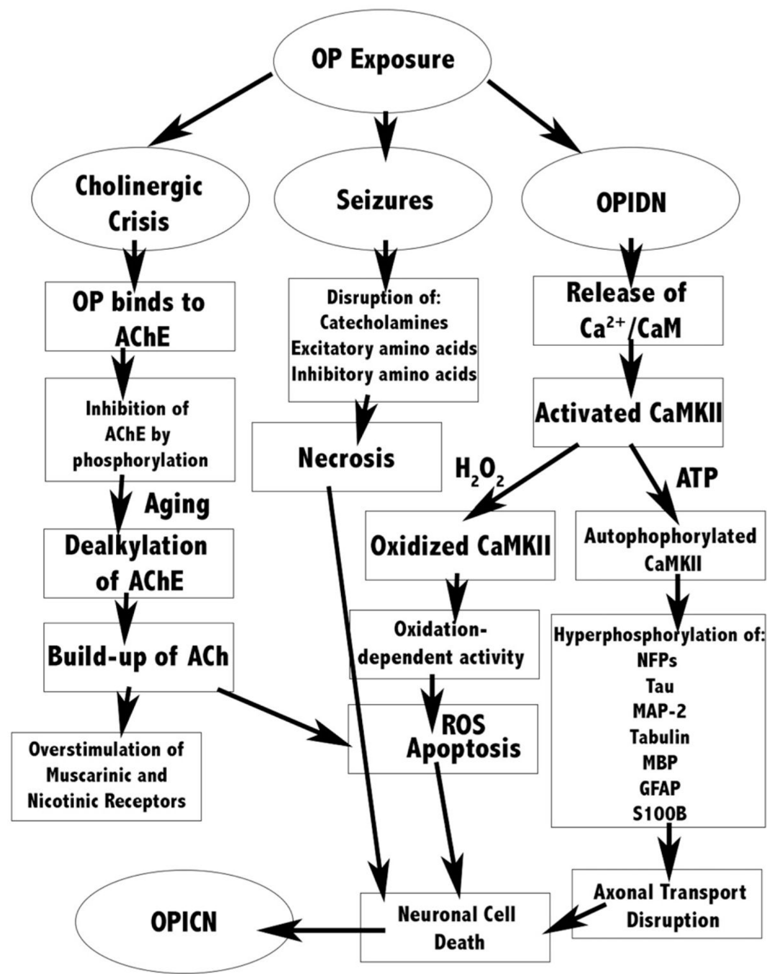

Figure 6.

Schematic presentation of the mechanisms of organophosphate poisoning. Abbreviations: OP: organophosphate; AChE: acetylcholinesterase; OPIDN: organophosphate-induced delayed neurotoxicity; NFPs: neurofilament protein; MBP: myelin basic protein; GFAP: glial fibrillary acidic protein; S100B: S100 calcium-binding protein B; OPICN: organophosphate-induced chronic toxicity; ROS: reactive oxygen species.

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Table 1

List of abbreviations, acronyms, and symbols.

Abbreviation	Full text
2-PAM	2-Pyridine aldoxime methyl chloride
5-HT	Serotonin
8-OhdG	8-Hydroxy-2′-deoxyguanoside
Ach	Acetylcholine
AChR	Acetylcholine receptor
AChE	Acetylcholinesterase
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ASP	Aspartate
Atg1	Autophagy protein 1
Atg5	Autophagy protein 5
ATP	Adenosine triphosphate
BBB	Blood brain barrier
BDNF	Brain-derived neurotrophic factor
BEAP	Brainstem auditory evoked potentials
BECN1	Beclin-1
BuChE	Butyryl cholinesterase
CA	Cornu Ammonis
CaMKII	Ca2+/calmodulin kinase II
ChE	Cholinesterase
CHL	Chlorisondamine
CI	Mitochondrial complex I
CNS	Central nervous system
CPF	Chlorpyrifos or *O,O*-Diethyl *O*-3,5,6-trichloropyridin-2-yl phosphorothioate
CWC	Chemical Weapons Convention
DA	Dopamine
DFP	Diisopropylfluorophosphate
DIMP	Di-isopropyl methylphosphonate
EBA	Endothelial barrier antigen
EEG	Electroencephalogram
EGF	Epidermal growth factor
FC	Frontal cerebral cortex
FGF-2	Fibroblast growth factor
FRAP	Ferric reducing antioxidant power
GA	Tabun: ethyl dimethylphosphoramidocyanidate
GABA	gamma-Amino-butyric acid
GB	Sarin: isoproyl methylphosphonofluoridate
GD	Soman: O-pinacolyl methylphosphonofluoridate
GF	Cyclosarin: cyclohexyl methylphosphonofluoridate
GFAP	Glial fibrillary acidic protein

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Abbreviation	Full text
GLU	Glutamate
GW	Gulf War
GWI	Gulf War Illness
HL-6	Diquaternary obidioxime
HPA	Hypothalamic-pituitary-adrenal
hPON-1	Human paraoxonase
IED	Improvised explosive device
IL	Interleukin
IM	Intramuscular
Kainic acid	2-Carboxy-3-carboxymethyl-4-isopropenylpyrrolidine
LCt_{50}	Lethal concentration and time
LD_{50}	Lethal dose 50%
M	Primary motor cortex
mAChR	Muscarinic acetylcholine receptor
nAChR	Nicotinic acetylcholine receptor
MAP	Microtubule-associated proteins
MINA	Monoisonitrosoacetone
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
NE	Norepinephrine
NFκB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NGF	Nerve growth factor
NMDA	N-Methyl-D-aspartate receptor
NTE	Neuropathy target esterase
OP	Organophosphate
OPCW	Organization of the prohibition of chemical weapons
OPICN	Organophosphate-induced chronic neurotoxicity
OPIDN	Organophosphate-induced delayed neurotoxicity
OPIED	Organophosphate-induced endocrine disruption
PBR	Peripheral benzodiazepine receptor
PGE	Prostaglandin
PMSF	Phenylmethylsulfonyl fluoride
PNS	Peripheral nervous system
Protexia®	Pegylated rBChE
PTSD	Post-traumatic stress disorder
RBC	Red blood cell
rBChE	Recombinant human BChE
rePON-1	Recombinant paraoxonase
TBARS	Thiobarbituric acid reactive substances
TCP	Tri-cresyl phosphate
TCR	T-cell receptor
TNF	Tumor necrosis factor

Abbreviation	Full text
TOCP	Tri-o-cresyl phosphate
USSR	Union of Soviet Socialist Republics
VEP	Visual evoked potential
VR	*O*-Isoprobpyl *S*-2-diethylaminoethyl methylphosphonathioate
VX	O-Ethyl S-[2-(diisopropylamino)ethyl] methylphosphonothioate
WAIS	Wechsler Adult Intelligence Scale
YT	Human natural killer-like cell line

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Table 2

IUPAC nomenclature, CAS No., and molecular formula of chemicals cited in the text.

Chemical	IUPAC name	CAS No.	Molecular formula
Atropine	Endo-(±)-α-(Hydroxymethyl)benzeneacetic acid 8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester	51-55-8	$C_{17}H_{23}NO_3$
Chlorisondamine	Trimethyl-[2-(4,5,6,7-tetrachloro-2-methyl-isoindolin-2-ium-2-yl)ethyl]ammonium dichloride	7701-62-4	$C_{14}H_{20}C_{l6}N_2$
Cyclosarin	Cyclohexyl methylphosphonofluoridate	77-81-6	$C_7H_{14}FO_2P$
Diazepam	7-Chloro-1-methyl-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one	439-14-5	$C_{16}H_{13}ClN_2O$
Diisopropylcarbodiimide	N,N′-Diisopropylcarbodiimide	693-13-0	$C_7H_{14}N_2$
Diisopropylfluorophosphate	Bis(propan-2-yl) fluorophosphonate	55-91-4	$[(CH_3)_2CHO]_2POF$
HI-6	1-[[[4-(Aminocarbonyl)pyridinio]methoxy]methyl]-2-[(hydroxyimino)methyl]-pyridinium chloride	34433-31-3	$C_{14}H_{16}N_4O_3 \cdot 2Cl$
Isopropyl alcohol	2-Propanol	67-63-0	C_3H_8O
Isopropyl amine	Propan-2-amine	75-31-0	C_3H_9N
Ketamine	2-(2-Chlorophenyl)-2-(methylamino)cyclohexanone	6740-88-1	$C_{13}H_{16}ClNO$
Levetiracetam	(2S)-2-(2-Oxo-1-pyrrolidinyl)butanamide	102767-28-2	$C_8H_{14}N_2O_2$
Methylphosphonyl difluoride	Methylphosphonic difluoride	676-99-3	CH_3POF_2
Phenylmethylsulfonyl fluoride	α-Toluenesulfonyl fluoride	329-98-6	$C_7H_7FO_2S$
Pralidoxime	2-Pyridine aldoxime methyl chloride	6735-59-7	$C_7H_9N_2O^+$
Procyclidine	1-Cyclohexyl-1-phenyl-3-(1-pyrrolidinyl)-1-propanol	77-37-2	$C_{19}H_{29}NO$
Propofol	2,6-Diisopropylphenol	2078-54-8	$C_{12}H_{18}O$
Soman	O-Pinacolyl methylphosphonofluoridate	96-64-0	$C_7H_{16}FO_2P$
Tabun	Ethyl dimethylphosphoramidocyanidate	77-81-6	$C_5H_{11}N_2O_2P$
TOCP	Tri-o-cresyl phosphate	1330-78-5	$C_{21}H_{21}O_4P$
Tributylamine	N,N-Dibutyl-1-butanamine	102-82-9	$C_{12}H_{27}N$
VX	O-Ethyl S-[2-(diisopropylamino)ethyl] methylphosphonothioate	50782-69-9	$C_{11}H_{26}NO_2PS$

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Table 3

World stockpile and destruction of chemical weapons.

Country	Declared stockpile (Schedule 1, Tons)	% OPCW verified destroyed	Date of full destruction	Destruction deadline
Albania	17 (20)	100	July 2007 (20)	N/A
India	1,044 (22)	100	March 2009 (21)	N/A
Iraq	Remnant munitions (31)	Not started (31)	–	N/A
Libya	25 (24)	100	January 14 (24)	–
Japan (In China)	–	Ongoing	–	2022 (Commitment) 32
Russia	40 000 (29)	92 (30)	N/A	29 April 2012 (Intends by December 2013) (28)
South Korea	3000–3500 (21)	100	July 2007	N/A
Syria	1040 (26)	100	August 2014	N/A
United States	33 600 (27)	90 (27)	N/A	29 April 2012 (Intends by December 2013) (28)

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Table 4

Sarin attacks against civilian populations.

Location	Date/time	Number of dead	Number of non-fatal injured	Chemical agent	Perpetuator
Matsumoto, Nagano, Japan	27/28 June 1994 11:00 p.m.–4:15 a.m.	8	>600	Sarin	Aum Shinrikyo
Tokyo Subway	20 March 1995 7:00–8:00 a.m.	12	5500	Sarin	Aum Shinrikyo
Halabja, Kurdistan, Iraq	16 March 1988	3200–5000	7000–10 000	Sarin, tabun, mustard gas	Iraqi Government
Ghouta, Syria	21 August 2013	1729	3600	Sarin	Syrian Government

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Table 5

Chemical properties of sarin (GB).

Name	Sarin	Source
Synonyms	GB	Merck
	Methylphosphonofluoridic acid 1-methylethyl ester	
	O-Isopropoxymethylphosphoryl fluoride	
	Isopropyl methylphosphonofluoridate **(MERCK)**	
CAS No.	107-44-8 **(CRC)**	CRC
PubChem	http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=7871	–
Molecular formula	$C_4H_{10}FO_2P$ **(CRC)**	CRC
Molecular weight (g/mol)	140.093 **(CRC)**	CRC
Composition	C 34.29%, H 7.19%, F 13.56%, O 22.84%, P 22.11%	CRC
Density	1.0887 g/cm^3 at 25 °C	MSDS
	1.102 g/cm^3 at 20 °C **(MSDS)**	
Melting point	–57 °C **(CRC)**	CRC
Boiling point (°F)	147 °C **(CRC)**	CRC
Water solubility	Miscible **(MSDS)**	CRC
State	Liquid **(MSDS)**	MSDS
Color	Colorless **(MSDS)**	MSDS
Odor	Odorless **(MSDS)**	MSDS
Vapor pressure (mm Hg at 77 °F)	2.9 **(MSDS)**	MSDS
Volatility (mg/m at 77 °F)	22 000 **(Merck)**	Merck
LCt_{50} (human mg-min/m^3)	100 **(Merck)**	Merck

The Merck Index: an encyclopedia of chemicals, drugs, and biologicals. 12th ed. In: Budavari S, O'Neal MJ, Smith A, Heckelman PE, Kinneary JF, editors. Whitehouse Station (NJ): Merck & Co.; 1996.

CRC handbook link: http://www.hbcpnetbase.com/.

MSDS: http://www.gulfweb.org/bigdoc/report/appgb.html#Physical%20Data.

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Table 6

LD_{50} of sarin in various species with different routes of exposure.

Organism	Route	Reported dose (normalized dose)
Human	Inhalation	90 μg/m^3 (0.09 mg/m^3) (Rengstorff 1985)
Human	Oral	102 μg/kg/3D-I (0.102 mg/kg) (Grob & Harvey 1958)
Human	Oral	2 μg/kg (0.002 mg/kg) (Grob & Harvey 1958)
Human	Intramuscular	30 μg/kg (0.03 mg/kg) (Grob & Harvey 1958)
Human	Skin	28 mg/kg (28 mg/kg) (ChemIDplus Advanced 2009)
Monkey	Intramuscular	22 300 ng/kg (0.0223 mg/kg) (ChemIDplus Advanced 2009)
Monkey	Intravenous	20 μg/kg (0.02 mg/kg) (ChemIDplus Advanced 2009)
Pig	Intravenous	15 μg/kg (0.015 mg/kg) (ChemIDplus Advanced 2009)
Pig	Skin	116 mg/kg (116 mg/kg) (ChemIDplus Advanced 2009)
Cat	Intravenous	22 μg/kg (0.022 mg/kg) (O'Leary et al., 1961)
Cat	Subcutaneous	30 μg/kg (0.03 mg/kg) (Goldstein et al. 1987)
Rabbit	Intravenous	15 μg/kg (0.015 mg/kg) (Wills 1961)
Rabbit	Skin	925 μg/kg (0.925 mg/kg) (ChemIDplus Advanced 2009)
Rabbit	Subcutaneous	30 μg/kg (0.03 mg/kg) (Gordon & Leadbeater 1977)
Rabbit	Intravenous	15 μg/kg (0.015 mg/kg) (Wills 1961)
Rabbit	Skin	925 μg/kg (0.925 mg/kg) (ChemIDplus Advanced 2009)
Guinea pig	Inhalation	128 mg/m^3/2 M (128 mg/m^3) (ChemIDplus Advanced 2009)
Guinea pig	Subcutaneous	30 μg/kg (0.03 mg/kg) (Coleman et al. 1968)
Hamster	Subcutaneous	95 μg/kg (0.095 mg/kg) (Coleman et al. 1968)
Rat	Intramuscular	100 μg/kg (0.10 mg/kg) (Abou-Donia et al. 2002)
Rat	Intramuscular	108 μg/kg (0.108 mg/kg) (ChemIDplus Advanced 2009)
Rat	Intramuscular	100 μg/kg (0.1 mg/kg) (Abou-Donia et al. 2002)
Rat	Intraperitoneal	218 μg/kg (0.218 mg/kg) (Fleisher et al. 1963)
Rat	Intravenous	39 μg/kg (0.039 mg/kg) (Fleisher et al. 1963)
Rat	Oral	550 μg/kg (0.55 mg/kg) (ChemIDplus Advanced 2009)
Rat	Skin	2500 μg/kg (2.5 mg/kg) (ChemIDplus Advanced 2009)
Rat	Subcutaneous	103 μg/kg (0.103 mg/kg) (Brimblecombe et al. 1970)
Rat	Aerosolized	154 μg/kg (2.5 mL ×60–80 breaths/min) (Collins et al. 2013)
Mouse	Inhalation	5 mg/m^3/30 M (5 mg/m^3) (Lohs 1960)
Mouse	Intramuscular	164 μg/kg (0.164 mg/kg) (ChemIDplus Advanced 2009)
Mouse	Intraperitoneal	283 μg/kg (0.283 mg/kg) (Van Meter & Karczmar 1968)
Mouse	Intravenous	109 μg/kg (0.109 mg/kg) (Tripathi & Dewey 1989)
Mouse	Skin	1080 μg/kg (1.08 mg/kg) (ChemIDplus Advanced 2009)
Mouse	Subcutaneous	60 μg/kg (0.06 mg/kg) (Lohs 1960)
Mouse	Intravenous	109 μg/kg (0.109 mg/kg) (Tripathi & Dewey 1989)
Mouse	Skin	1080 μg/kg (1.08 mg/kg) (ChemIDplus Advanced 2009)
Mouse	Subcutaneous	60 μg/kg (0.06 mg/kg) (Lohs 1960)
Chicken	Subcutaneous	16 673 μg/kg (16.673 mg/kg) (Gordon et al. 1983)
Hen	Oral	100 μg/kg (0.1 mg/kg) (Abou-Donia et al. 1997)

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Table 7

Actions of sarin exposure.

Effect of sarin exposure	Symptoms		
	Acute	**Delayed**	**Chronic**
Biochemical	Irreversible inhibition of AChE at muscarinic and nicotinic receptors (Cannard 2006)	Hyperphosphorylation of cytoskeletal proteins such as α- and β-tubulin and MAP-2 (Abou-Donia et al. 1993) Increased CaMKII expression and activity (Damodaran et al. 2006b)	Sarin and PB induce Oxidative stress (Abu-Qare and Abou-Donia 2001)
Metabolism	*In vitro* metabolism of PB, DEET, permethrin (Abu-Qare & Abou-Donia 2008)	–	–
Gene Expression	Sarin and mRNA for GFAP (Damodaran et al. 2002) Sarin and alterations in mRNA for AChE (Damodaran et al. 2003)	–	Sarin-induced alterations of Gene expression (Damodaran et al. 2006a, 2006b)
Pathological	Muscle weakness (Karalliedde et al. 2000) Acute sarin exposure Khan et al. 2000 Muscle twitching (Karalliedde et al. 2000) Fasciculation (Karalliedde et al. 2000) Smooth muscle contraction (Cannard 2006) Miosis (Cannard 2006) Ciliary spasm (Cannard 2006 #66; Abdel-Rahman et al. 2002 #179; Abdel-Rahman et al. 2002 #433) Tearing (Cannard 2006) Rhinorrhea (Cannard 2006) Vomiting (Cannard 2006) Abdominal cramps (Cannard 2006) Urinary and bowel incontinence (Cannard 2006) Bronchoconstriction (Cannard 2006) Excess bronchosecretions (Cannard 2006) Wheezing (Cannard 2006) Central apnea (Cannard 2006) Respiratory paralysis (Cannard 2006) Bradyarrhythmias (Cannard 2006) Hypotension (Cannard 2006)	Weakness, numbness and atrophy in legs and feet; OPIDN (Abou-Donia 1981) Weakness in hands (Abou-Donia 1981) Symmetrical flaccid paralysis (Abou-Donia 1981) Subchronic sarin exposure Jones et al. 2000	Sarin induced OPICN (Abou-Donia 2003)
Structural	Permeabilization of blood brain barrier (Abu-Qare & Abou-Donia 2002) Neuronal degeneration in cerebrum, brain stem, midbrain, cerebellum (Abu-Qare & Abou-Donia 2002)	Axonal swelling and degeneration (Abou-Donia & Lapadula 1990) Wallerian-type degeneration in central and peripheral nervous systems (Abou-Donia & Lapadula 1990)	Neuropathological alterations in cortex, hippocampal formation, cerebellum, piriform cortex and thalamus (Hulet 2002; Grauer et al. 2008) Distonal axonal peripheral neuropathy (Pilkington 1999) Decrease in volume of white matter, right lateral ventricle and left lateral ventricle (Heaton et al. 2007)
Neuropsychological	Headache (Cannard 2006) Depression (Cannard 2006) Anxiety (Cannard 2006) Irritability (Cannard 2006) Fatigue Insomnia (Cannard 2006) Nightmares (Cannard 2006) Inability to concentrate (Cannard 2006) Memory loss (Cannard 2006) Impaired judgment (Cannard 2006) Seizure (McDonough & Shih 1997; Cannard 2006) (McDonough & Shih 1997; Cannard 2006)	Sarin PB sensorimotor (Abou-Donia et al. 2002)	Fatigue (Yokoyama et al. 1998a, 1998b) Decreased psychomotor performance (Yokoyama et al. 1998a, 1998b) Memory disruption (Nishiwaki 2001 #54) Decrease in visuospatial ability (Proctor et al. 2006)