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A Review of Chemical Warfare Agents Linked to Respiratory and Neurological Effects Experienced in Gulf War Illness

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Abstract

Over 40% of veterans from the Persian Gulf War (GW) (1990-1991) suffer from Gulf War Illness (GWI). Thirty years since the GW, the exposure and mechanism contributing to GWI remain unclear. One possible exposure that has been attributed to GWI are chemical warfare agents (CWA). While there are treatments for isolated symptoms of GWI, the number of respiratory and cognitive/neurological issues continues to rise with minimum treatment options. This issue doesn't only affect veterans of the GW, importantly these chronic multisymptom illnesses are also growing amongst veterans who have served in the Afghanistan-Iraq war. What both wars have in common are their regions and inhaled exposures. In this review, we will describe the CWA exposures such as sarin, cyclosarin, and mustard gas in both wars and discuss the various respiratory and neurocognitive issues experienced by veterans. We will bridge the respiratory and neurological symptoms experienced to the various potential mechanisms described for each CWA provided with the most up-to-date models and hypotheses.

Keywords

Chemical Warfare Agents; Sarin; Cyclosarin; Sulfur Mustard; Gulf War Illness; Chronic Multisymptom Illness; Respiratory; Neurological effects; Organophosphorus compounds; Nitrogen Mustard; CEES

Introduction

In August 1990, over 700,000 U.S. troops were deployed for the Persian Gulf War (GW) to serve in combat missions: Operation Desert Shield and Desert Storm[1]. Upon returning from the GW, veterans experienced a collection of chronic multisymptomatic health complications within a year[2] . These symptoms included fatigue, headache, memory problems, muscle/joint pain, diarrhea, dyspepsia/indigestion, terminal tumors,

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skin conditions, arthritis/ joint issues, gastrointestinal problems, respiratory complications, post-traumatic stress disorder, and chronic fatigue syndrome[2]. The combination of these symptoms is better known today as Gulf War Illness (GWI) or Chronic Multisymptom Illness (CMI).

Diagnosis Criteria for Gulf War Illness

GWI is diagnosed using either the Center for Disease Control's (CDC) CMI criteria or the Kansas GWI Criteria. The CDC CMI criteria require that veterans have two of the following chronic symptoms, lasting for more than six months: pain, fatigue, mood swings, and alterations to cognition[3,4]. The Kansas GWI criteria is the stricter of the two, defined as having at least one chronic symptom in three of the following classifications: fatigue/ sleep problems, pain, neurologic/cognitive/mood symptoms, gastrointestinal symptoms, respiratory symptoms, and skin symptoms[3,4]. The U.S Department of Veteran Affairs (VA) and the Department of Defense (DoD) are working together to merge both these criteria to create uniform guidelines for clinical and research practices. Currently, the VA guidelines for diagnosing GWI are based on the time and location of service during exposure, types of exposure, potential exposures contributing to symptoms, and symptoms persisting for at least six months resulting in a disability rating of 10% or more[5].

Prevalence of Chronic Respiratory and Neurological Symptoms of GWI

Approximately 40% of veterans who served in the GW have been diagnosed with GWI[3,4]. GWI progression has been well documented in the Ft. Devens Cohort (FDC), one of the longest studied GWI cohorts (1991)[6]. This longitudinal study compared the prevalence of nine chronic medical conditions in 2013 and 2014[6]. The study results found a significant association between self-reported chemical/biological weapons exposure and chronic diseases such as asthma and bronchitis[6,7]. FDC men were approximately two times more likely (OR=1.987) to report having asthma compared to the general population (National Health and Nutrition Examination Survey; NHANES) and over three times more likely (OR = 3.175) to report chronic bronchitis compared to the general population as of 2013[6].

In addition to respiratory issues, veterans also reported an array of neurological problems that have been associated with exposures during the GW [8–10]. GW veterans also have a higher incidence of amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), brain cancer, repeated seizures, neuritis, chronic migraine headaches, chronic cognitive disorders, and mood-related problems compared to the general population[11–14]. Thirty- years since the GW, the VA has seen a rise in GWI diagnosis (25% to ~35%), and a rise in veterans experiencing various respiratory and neurological complications[2,4,5,15].

Sex differences and sex-specific effects have been observed in GWI sufferers. Male GW veterans have reported higher rates of sexual dysfunction and genital and bladder problems than non-GW veterans [16] . Female veterans' health outcomes have been examined using the GW veterans from the VA Cooperative Studies Program 585 GW Era Cohort and Biorepository (GWECB)[6,17,18]. In 2016, the GWECB found that GW-deployed women were significantly more likely to report cognitive (17% excess prevalence rate), neurological (15%

excess prevalence rate), mood (20% excess prevalence rate), and respiratory issues (19% excess prevalence rate) compared to non-deployed GW-era women veterans[6,17]. Women GW veterans also have higher rates of birth defects in their children and other reproductive issues than their non-GW veteran counterparts [19]. There is evidence that sex differences exist in GWI pathology and in the mechanisms driving it. GW-deployed women had a higher prevalence of most GWI symptoms than GW-deployed men, along with having higher rates of mild-to-moderate or severe GWI [17]. Plasma lipid profiles differ significantly between male and female veterans with GWI [20]. Plasma levels of central nervous system (CNS) autoantibodies were more elevated in men with GWI than in women with GWI [21]. Homeostatic modeling using data from veterans with GWI indicated that men and women with GWI have different homeostatic states, with men having hypercortisolism, low testosterone, and a disposition towards Th1 immune activation while women had hypocortisolism, high estrogen, and a disposition towards Th2 immune activation [22] . Sex differences also appear in animal models of GWI. A mouse model of GWI saw more cognitive impairment and increased CNS immune cell activation and inflammation in females compared to males [23]. Interestingly, this same study saw more greatly increased markers of peripheral inflammation in males than in females [23]. Another study using mice found that pyridostigmine bromide (PB), a reversible acetylcholinesterase inhibitor used in the Gulf War as an anti-nerve agent drug [24], increased fecal pellet production and delayed colonic transit in females, but not males [25]. This study also found that prior exposure to PB altered the effects of later treatment with palmitoylethanolamide, an anti-inflammatory, in sex-specific ways, with females experiencing an increase in myenteric neurons and muscarinic M3 receptors while males did not [25]. The neuroimmune effects of PB exposure were found to be more immediate in males, but delayed in females in a mouse model of GWI [26]. These animal studies have been summarized in Table 1..

Over 30 years after the GW, scientists have yet to understand the etiology of GWI. Multiple exposures have been implicated, particularly inhaled toxicants, such as depleted uranium (DU), pesticides (organophosphates and carbamates), fine particulate matter (PM) (building fires, oil well fires, and desert dust), and chemical warfare agents (sarin and sulfur mustard)[24]. Other non-respiratory exposures that have been suspected of contributing to the disease etiology include microbiological exposures, immunizations against anthrax, plague, botulism, and physiological stress components (i.e., sleep deprivation, extreme temperature, and combat stress)[31–33]. This review will focus on the etiology of GWI/CMI, focusing on chemical warfare agent (CWA) inhalation exposures suggested in disease pathology. For these exposures, this review will summarize the prevalence and likelihood of veterans being exposed, mechanistic studies, and the environmental factors that can cause neurological and lung inflammation resulting from inhaling CWAs.

The following terms were used during the literature search: “gulf war illness sex”, “gulf war children”, “gulf war illness reproductive”, “gulf war illness reproduction”,“GWI reproductive”, “gulf war illness”, “civilians chemical weapons gulf war”, “civilian exposure chemical weapons gulf war”, “civilian exposure chemical weapons”, “sarin Afghanistan”, “chemical warfare Afghanistan”, “chemical weapons Afghanistan”, “chemical exposure Afghanistan”, and “organophosphate inflammation”.

Chemical Warfare Agents

Soldiers were exposed to CWAs throughout both the Gulf War. CWA exposures included organophosphate (OP) compounds such as sarin (GB), cyclosarin (GF), diisopropyl fluorophosphate (DFP), and mustard gas (sulfur mustard; SM)[28,34,35]. During the time of Desert Storm, Iraq had an active chemical warfare program with vast amounts of munitions containing CWAs and bulk reservoirs located throughout the country[35]. Surprisingly, most exposures to these chemicals did not come from active combat; instead, after the war ended in March 1991, when U.S. soldiers destroyed the stockpiles of the Iraqi CWAs at the Khamisiyah munitions depot[34,35]. During this time, U.S troops also bombed various CWA research, storage, and mass production sites[34].

Much of the evidence suggests that troops were exposed, and many of the civilians who lived nearby were also affected by CWAs in the atmosphere. Shortly after known CWA exposures, military personnel and civilians reported respiratory complications and neurological symptoms due to the inhalation exposures from previously mentioned incidents[27,30,35].

Inhalation Exposure to Sarin (GB) and Cyclosarin (GF)

An estimated 99,000 U.S troops were potentially exposed to a mixture of GB and GF[34]. It is believed that the long-term effects of GB and GF inhalation could play a contributing role in the respiratory and neurological issues manifested in GWI. The munition dump filled with GB and GF, located in Khamisiyah, Iraq, was destroyed at a time when the highest number of U.S. troops were in the area and likely exposed to varying levels of the CWA mixtures[10,34]. Five years after the initial event, the DoD developed weather and wind schematics of the affected area to determine the number of troops affected and the potential concentration of the exposure[37]. Considering the size of the plumes from the destruction of the munition dump paired with the initial weather mapping, new maps were created seven years since the exposure and suggested that over 100,000 U.S. troops were exposed[38].

Organophosphorus Chemical Warfare Agent Mechanism

GB and GF are OP compounds known for inhibiting acetylcholinesterase (AChE)[39,40]. AChE's primary purpose is to hydrolyze acetylcholine (ACh) at the synapse of neuromuscular junctions[41]. Inhibition occurs when GB and GF phosphorylate the serine hydroxyl group on the active site of AChE. The inhibition of AChE will result in rapid accumulation of ACh, producing overstimulation at cholinergic synapses, often referred to as cholinergic crisis[40,42]. Two major classes of cholinergic receptors are the nicotinic and muscarinic receptors. Nicotinic receptors are found at the neuromuscular junctions, autonomic ganglia, and some parts of the CNS[43]. In contrast, muscarinic receptors are found on myocardial muscle, throughout the lungs, and in regions of the CNS[44].

Like with most organophosphorus compounds, the process of "aging", or the dealkylation of AChE when inhibited by OP compounds and subsequently becoming unable to be directly reactivated by pyridinium oximes (one of the main treatments for OP-poisoning), will occur with GB and GF if left untreated[45]. Once phosphorylated, the active site of AChE loses

an alkyl group, making it resistant to hydrolysis, blocking dephosphorylation. This aging process after GB and GF exposures can occur within 5 h of exposure[34]. GB and GF's mild to moderate toxicity results in pinpoint pupils, blurry vision, rhinorrhea, shortness of breath, chest tightness, and muscular weakness[10,34,39,40]. In comparison, severe symptoms can lead to seizures, bronchoconstriction, lung edema, and respiratory failure/pulmonary collapse leading to death due to the overload of ACh on the autonomic nervous system[39].

Many GW veterans experienced acute cholinergic syndrome, which was noticeable seconds to hours after initial exposure, presenting with previously mentioned mild to moderate toxicity symptoms[34,46]. Many low-level exposures can lead to acute cholinergic syndrome and should resolve within days. There is also evidence indicating a non-cholinergic pathway by which OP compounds exert their neurotoxic effects. Studies with organophosphate exposure in various models have been summarized in Table 2. Terry Jr *et al.* found that DFP exposures that did not produce cholinergic distress could still result in cognitive impairment in rats[49]. Locker *et al.* studied the effects of multiple GW-relevant AChE inhibitors, including the OP compounds DFP and chlorpyrifos-oxon (CPO), on neuroinflammation under stress conditions[50]. In this study, it was observed that only the OP AChE inhibitors caused significant neuroinflammation under either stressed or on-stressed conditions. Even more interestingly, it was found that the stressed condition both exacerbated neuroinflammation and reduced AChE inhibition in the OP-treated mice [50]. While the authors were not able to point out a specific non-AChE pathway responsible for the observed neuroinflammation, they did reject the role of AChE and postulated that phosphorylation by OP compounds may be a potential cause [50]. Miller *et al.* found similar results, with stress conditions exacerbating OP compound-induced neuroinflammation and markers of neuroinflammation generally not correlating with ACh activity in multiple regions of the brain[51]. The possibility that phosphorylation by OP compounds could be a causal factor of neuroinflammation was also mentioned by O'Callaghan *et al.* [52]. Yates *et al.* found that DFP plus cortisol in human-induced pluripotent stem cells (hiPSC's) resulted in alterations in tau levels, microtubule acetylation, and mitochondrial function. The authors also found that DFP plus cortisol in rats increased total tau in the CA3 region of the hippocampus, but nowhere else, and reduced total cell number without altering cell morphology in the CA3 region of the hippocampus[53]. Lein *et al.* treated guinea pigs with OP pesticides at doses that did not result in cholinergic intoxication, but did exacerbate vagally induced bronchoconstriction and bradycardia, leading the authors to conclude that OP compounds were acting directly on the muscarinic receptors. These effects were seen at doses that did not inhibit AChE activity, which, combined with the inability of the OP compounds at the doses given to exacerbate ACh-induced bronchoconstriction, indicates that OP compounds may exert their effects on the respiratory system in a manner independent of AChE[54]. Further evidence that OP compounds can act directly on muscarinic receptors is provided by Katz and Marquis, who found that paraoxon inhibits tritiated quinuclidinyl benzilate, [^{3}H]QNB, an antagonist of cholinergic muscarinic receptors[55]. While some OP compounds can act directly on muscarinic receptors at doses that do not inhibit AChE, OP compound that act directly on nicotinic receptors only do so at doses that also inhibit AChE[56]. Other possible mechanisms of OP-induced toxicity include inhibition of neurotoxic esterase and inhibition of ACh synthesis via reduced cAMP resulting in reduced choline uptake[56].

Inflammation has been seen in both animal and human models of OP exposure. Increased expression of proinflammatory cytokines in the CNS has been observed upon OP exposure in mice[50,52]. Guignet *et al.* found evidence of reactive astrocytes, microgliosis, and increased oxidative stress in the brains of rats exposed to OP compounds[57]. An observational study of agricultural workers exposed to OP pesticides found elevated levels of TNF-α (tumor necrosis factor), interleukin-6 (IL-6), and C-reactive protein in their blood[58]. Oral exposure to chlorpyrifos in a mouse model resulted in inflammation in the liver and altered gut microbiota[59]. OP compound exposure in a mouse model was also seen to produce lung inflammation as well as an exacerbated allergic response in ovalbumin (OVA)-sensitized mice[60].

Inhalation effects of GB and GF on the Respiratory System

The primary route of exposure causing the most toxic effects to GB and GF is via inhalation, but victims can also be exposed via dermal and ocular routes[61]. GB and GF trigger airway hyperreactivity through the inhibition of AChE, which in turn decreases the hydrolysis of ACh that is readily available[62]. By decreasing hydrolysis, ACh will accumulate on the cholinergic receptors, such as the muscarinic receptors. In the lung, muscarinic receptors are present throughout the airways and on epithelial cells[63]. In addition, the parasympathetic nerves that are innervated throughout the lung are known for releasing ACh onto the muscarinic receptors[64]. Given that the lung is one of the primary routes of exposure to GB and GF, victims of such exposure will experience respiratory symptoms. Still, it is unclear if exposure contributes to long-term effects observed in GWI.

Stemming from the vagus nerve, parasympathetic nerves are responsible for most of the lung's autonomic functions, such as airway contraction and relaxation. Normal contraction and bronchoconstriction result from the parasympathetic nerves releasing ACh onto the muscarinic receptors[65]. The severity of GB and GF exposures is highly dependent on the exposure dose. Less severe, acute toxicity to GB (ECt_{50}=1.0 mg-min/m^3) and GF (ECt_{50}=0.5 mg-min/ m^3) leads to increased nasal discharge, bronchoconstriction, wheezing, and increased mucus secretions[64]. As a result of the GB/GF ECt_{50}'s mentioned above, the overload of ACh will cause bronchoconstriction through the persistent activation of specific muscarinic receptors such as the M3 receptors on smooth airway muscles[64]. In severe cases, respiratory distress is likely to occur at concentrations higher than the ECt_{50} but below the LCt_{50} for GB (35 mg-min/m^3) and GF (35 mg-min/m^3)[64]. In more severe cases, respiratory distress can lead to death associated with the prolonged inhibition of AChE and cholinergic overload in the autonomic nervous system. This overload causes continual muscle contraction in essential organs such as the lungs (bronchospasms) and the heart (tachycardia) to the point of paralysis in the diaphragm and intercostal muscles[64]. Minimum information is available on long-term damage to lungs in humans due to the toxic nature of these compounds and their ability to predominantly affect the CNS.

Research is sparse in addressing the long-term respiratory effects of GB and GF exposure. It has been reported in rodent models that a one-time high dose inhalation exposure to GB (51.2 mg/m^3 for 15 min) did not produce lasting inflammatory effects in the lung and was cleared 2-3 weeks after initial exposure[66]. In another study, examining whether

subclinical doses had lasting respiratory impacts that closely resemble GW exposures, a 5-day repeated exposure to a subclinical dose of 0.4 mg/m^3/h/day (nose-only exposure) of GB was administered to rats to determine inflammation in the lung[67]. While changes in gene expression of proinflammatory cytokines TNF-α, interleukin-1β (IL-1β), and IL-6 were significantly increased as well as inhibition of T-cell function, however, these inflammatory findings did not persist beyond two weeks[67,68]. Despite the lack of evidence in humans, much of the animal model data indicates acute short-term respiratory damage but fails to confirm long-term damage. This topic merits further exploration to determine potential long-term synergistic effects of acute injury experienced from exposure to GB/GF in addition to other exposures that potentially contribute to the respiratory impacts observed in GWI.

Inhalation Effects of GB and GF on the Central Nervous System

Though neurocognitive symptoms and brain alterations don't occur until months or even years after initial exposure to GB and GF, acute neurological effects follow immediately after initial exposure, such as seizures, paralysis, and in severe cases, death[69]. The enzyme AChE is essential in function of the autonomic nervous system and within the autonomic nervous system reside the parasympathetic nerves, as previously mentioned. By inhibiting AChE, nerve signaling occurs indefinitely, overloading cholinergic receptors with ACh[62]. The continuous firing of these nerve signals leads to continual muscle contractions throughout the body, causing paralysis, respiratory failure, tachycardia, and death if left untreated or exposed to a high concentration[70,71]. Once AChE inhibition subsides, the neurocognitive decline becomes more apparent in affected veterans, such as attention, memory, and learning[10,72–74]. In addition to neurocognitive decline, there have been several reports of brain tissue changes, predominantly in the hippocampus, resulting from GB and GF exposures that are detected months to years after exposure[75,76].

Many studies have focused on how GB and GF exposure affects the brain structures and their potential involvement in neurocognitive decline experienced by veterans with GWI. The brain structure affected by inhalation of GB and GF is the hippocampus region of the brain[75,76]. The hippocampus is located between the temporal lobes and comprises of the dentate gyrus and what is known as Ammon's horn (cornu ammonis). The role of the hippocampus involves receiving and processing information that contributes to learning and memory formation along with spatial navigation[77]. Given the hippocampus function, veterans who disclosed being near the plumes of CWA have reported a decline in dexterity, visuospatial functions, and memory[78–80]. Similarly, reduction in white matter and hippocampal volume was also found in veterans, which was associated with the decline in neurocognition as mentioned earlier[81]. Fourteen to nineteen years after the GW, veterans took part in GWI research at the San Francisco Veterans Affairs Medical center between 2005 and 2010. These Veterans had a significant reduction in hippocampal volume and gray matter volume (observed through 1.5T and 4T magnetic resonance imaging) as well as notable changes on the continuous performance test (measuring attention) (n=56 exposed/unexposed)[75]. These findings correlate with known GB attacks victims such as the 1995 Tokyo terrorist attacks, who also notably had smaller hippocampal volume than those unexposed[82].

In further understanding the mechanism behind GB/GF exposure in the brain, animal models have been used to replicate the neurological symptoms in GWI. In rodent models, a one-time exposure to GF (1.6-5.2 mg/m^3) caused a drastic decrease in learned behavioral tasks but was recovered months after[83]. In characterizing the effect of GB on the hippocampus, a metabolomic study examined the impact of a low dose exposure of GB [0.4 x LD_{50}= 16.8 μg/kg via subcutaneously (*s.c.*)] and found that phospholipid and sphingolipid metabolism was disrupted in the hippocampi of guinea pigs after 14 days[76]. In studying the effects of GB on the brain, an OP surrogate known as DFP has been routinely used[84]. DFP has made it possible to recapitulate many neurological effects observed in GWI in rat models. In a study done in rats, 400 mg/kg of DFP, a dose authors said to be comparable to what GW soldiers experienced in the field, was administered *s.c.* over five days[85]. Three months after DFP exposure, rats had several cognitive issues such as anxiety, chronic depression, and memory problems that persisted along with neuronal cell death in the hippocampus[85]. Although different neuronal populations within the hippocampus have been attributed to memory issues, it has also been hypothesized that Ca^{2+} fluctuations may contribute to synaptic plasticity leading to the persistent memory issues exhibited in GWI[86]. The further evaluation of the Ca^{2+} hypothesis in neurological morbidities can provide a relevant therapeutic target. While the information provided is advantageous in elucidating a mechanism of GB/GF in the brain, most studies lack an appropriate route of exposure (*s.c.* injections versus inhalation exposure) which is critical in understanding the manifestation of GWI.

The Progression of Therapeutics for GB and GF Exposures

Some of the most commonly used therapeutics for OP poisoning are atropine, oxime, and diazepam. As an anticholinergic, atropine blocks the effects of the ACh overload on muscarinic receptor regions[62]. In addition to atropine, oximes are often used to reactivate AChE, aiding in regaining respiratory functions[40]. The usage of oximes such as pralidoxime as a treatment is controversial due to timing and mechanism in alleviating respiratory distress[87]. There are a couple of conditions where oxime treatments are ineffective and detrimental to victims, such as the aging of AChE and the rate of AChE reactivation in response to the oxime is slower than the rate of bond formation between OP and AChE[40,87]. Paired with atropine and oxime usage, the anticonvulsant diazepam alleviates victims of seizures following such exposure[88]. Although these therapeutics are effective when the exposure is known, and the victim can seek medical attention immediately, timing is a huge factor in their effectiveness. Due to the above limitations of veterans not knowing when and what they were exposed to, alternative therapies are being explored for OP poisoning concerning GB/GF exposures that encompass catalytic bio-scavengers, phenobarbital, urethane, neurosteroids, and ketamine.

An alternative therapeutic currently being studied in animals are catalytic bioscavengers. Bioscavengers are a novel approach, with their ability to efficiently eliminate nerve agents such as GB/GF by catalyzing their hydrolysis, entirely inhibiting toxicity[89,90]. Bioscavengers would be used prophylactically and could render benefits if soldiers anticipate an attack[89,90]. Its limitation resides in the fact that administration after exposure would be useless. There needs to be enough bioscavengers in the body before an exposure to eradicate GB/GF efficiently. A bioscavenger of interest is human butyrylcholinesterase

(BChE)[91,92]. Human butyrylcholinesterase is a non-specific cholinesterase enzyme made in the liver and is found in plasma[92]. Large doses of BChE must be readily available in the blood to catalyze lethal concentrations of nerve agents, making it a less viable option[91,92]. However, it is possible to improve efficacy, as site-directed mutagenesis of BChE has shown promising results as a viable prophylactic option. The G117H mutation has yielded exciting results against GB, catalyzing the reaction 174-fold higher than wild-type BChE, but this remains insufficient to implement as a treatment[91,93]. This is a significant limitation of this bioscavenger as a therapeutic, and the search for a bioscavenger with a higher catalytic reaction rate is underway.

Since the discovery of BChE, other bioscavengers have been identified, including mammalian paraoxonase (PON1) and diisopropylfluorophosphatase (DFPase) from *Loligo vulgaris*[89,94,95]. PON1 is a lipoprotein secreted by the liver, but local synthesis is said to occur in most tissues in the body. Intravenous (*i.v.*) injection of a chimeric PON1 mutant, IIG1, at 0.2 and 1.0 mg/kg, 60 min before *s.c.* injection of GF (100 μg/kg), completely prevented signs of poisoning and lethality in guinea pigs[96]. While the PON1 mutant, IIG1, shows some promise as a prophylactic treatment in *in vitro* and *in vivo* studies, more research is needed to determine efficacy in humans.

DFPase, originating from squid (*Loligo vulgaris*), is another potential bioscavenger. Unlike previously mentioned bioscavengers, the DFPase enzyme's non-human origin causes it to readily clear the body faster and poses a potential risk for unwanted immune responses[97]. To make this enzyme more favorable, polyethylene glycol (PEG) can be added to solve the issues mentioned. This concept was tested using a similar OP nerve agent, Soman. It was found that dosing rats with 71 mg/kg PEGylated DFPase (considered extremely high) 5 minutes before Soman (3 x LD_{50}) *s.c.* kept the rats alive while lower doses did not[97]. While the higher dose of PEGylated DFPase kept the rats alive, the doses are not biologically relevant unless an increase in substrate affinity allows for the dosage to be substantially lowered.

A prominent effect of OP's (GB, GF, and DFP) are the seizures and refractory status epilepticus (SE) following exposure. While diazepam is one of the main treatments to treat seizures and SE after OP exposure, victims are sometimes unresponsive[88,98]. Due to the failure of diazepam, drugs such as phenobarbital and urethane have been studied for therapeutic consideration. In rats, SE was induced by a *s.c.* administration of 3.2 mg/kg DFP[99]. Forty minutes after DFP exposure, phenobarbital was administered at 30,60, and 100 mg/kg[99]. At 100 mg/kg of phenobarbital, seizure activity was terminated entirely, and there was significant neuroprotection in the brain regions associated with SE[99]. However, there are benefits and inherent risks in using phenobarbital, such as an unconscious state or death.

Urethane, which was formerly used as an anesthetic, is presently an anticonvulsant. Electroencephalography on DFP exposed rats (0.8 urethane administered 1 h after a 5mg/kg DFP) showed that urethane treatment terminated SE and prevented future seizures[100]. Urethane has also been shown to robustly attenuate neurodegeneration and inflammatory mediators (IL-1β and TNFα) at 24 h after DFP administration in rats[101]. Another drug under investigation for treating seizures and SE after OP exposure is the neurosteroid

pregnenolone. Dual therapy with pregnanolone (4 mg/kg) and diazepam (10 mg/kg) has been shown to reduce experienced SE time in rats exposed to GB and decreased neuronal degeneration months after a 60-min one-time exposure of 3.0 LCt_{50} of GB[102].

While seizures and SE remain significant OP symptoms of poisoning, another considerable concern is the effects OP has on mood and the chronic fatigue experienced by veterans[103,104]. GB/GF are thought to be underlying factors that play a role in mood dysfunction seen in veterans. A study utilizing a DFP exposed rat model showed that when treated with ketamine (3, 5, and 10 mg/kg, *i.p.*), rats exhibited significantly improved behavioral signs as early as 1 h post-administration, persisting to 24h[105]. Many of these treatment strategies have yielded promising results that need further investigation. GWI remains a significant threat to countless veterans' health and quality of life. While many of the discussed treatment strategies here have yielded promising results, we must direct additional resources to elucidate these mechanisms to understand and counteract the progression of these disorders.

Inhalation Exposure to Sulfur Mustard

Along with GB and GF, Sulfur mustard (bis(2-chloroethyl) sulfide; SM) is a CWA identified in destroyed munitions in Khamisiyah, Iraq, as well as Muammadiyat Ammunition Storage Site[46,106]. The DoD recorded over 15 metric tons of SM at the Muammadiyat Ammunition destruction site alone[46,106]. In Khamisiyah, the amount of mustard gas was reported to be far less than the nerve agents, GB and GF, but troops near the site still ran the risk of exposure[35]. DoD estimates over 4,000 soldiers have been acutely exposed to high concentrations of mustard gas. In contrast, the remaining troops in neighboring areas could have been exposed to lower concentrations due to wind patterns[35,46]. This estimation is expected to be much higher according to government documents, but an exact number remains unknown[46]. SM is thought to cause long-term respiratory and neurological damage, as seen in veterans throughout different wars that experienced this exposure. An overview of the symptoms experienced following SM exposure is detailed in Figure 1 and the animal studies utilizing SM and analogues have been summarized in Table 3..

Mechanism of Action for Sulfur Mustard

SM is a bifunctional vesicating agent known for causing severe blistering and burns on the skin, eyes, and most notably, the respiratory tract[107,108]. Despite popular belief, SM is not a gas; it is an oily liquid aerosolized when used on the battlefield, giving it a "gas-like" appearance[109]. For this reason, one of the most common modes of exposure is inhalation. Many mechanisms of toxicity have been proposed, but to date, no mechanism fully encapsulates the systemic impairment and damage experienced as a result of SM inhalation. The lack of a particular mechanism has made developing a viable treatment for SM exposure challenging. Below we will discuss some of the significant mechanisms highlighted in the literature that contribute to SM-mediated toxicity.

Alkylation Events: One of SM's first and most studied cytotoxic chemical reactions is DNA alkylation. Biologically, SM will form sulfonium ions that will rapidly undergo another reaction, the opening of the sulfonium ring, forming carbenium ions[109]. Carbenium

ions are then readily available to interact with DNA[107]. Approximately 61% of alkylation events occur on the guanine at the N-7 position, and 8% of alkylation events occur on an adenine at the N-1 position forming adducts[110,111]. SM can react with other nucleotides at various positions, but these are less common[110]. Once these alkylation events occur on one side of the DNA strand, due to SM's bifunctional characteristics, it can react once more, forming a cross-linkage (Figure 2)[110,111]. This cross-linkage reaction between guanines/adenines of the double helix molecule disrupts cellular metabolism and alterations in the DNA replication mechanism[110]. It has been estimated that about 17% of these alkylation events will form inter-strand links causing DNA strand breaks, in turn inhibiting cellular function such as replication, and ultimately causing mutations (cancer formation) or cell death[110,112]. This is especially detrimental to fast-dividing cells such as precursor immune cells found in the bone marrow. The death of these cells will directly affect immune responses to infection, as an example. SM can form adducts with other molecules aside from DNA, such as RNA, proteins, and lipids (Figure 2)[113]. SM's ability to alkylate different biological components is voluminous when diving into the damage and cellular impairment SM causes. Still, the double-strand cross-linkage only occurs in about 17% of events which doesn't account for the remaining damage SM causes. It is essential to understand that DNA alkylation, either single or double strand cross-linkage event, activates downstream mechanisms that are believed pivotal to SM-induced injury.

In the formation of DNA adducts, poly(ADP-ribose)polymerase type1 (PARP-1) is activated. PARP-1 is an essential protein in the DNA base excision repair mechanism[113–115]. In the event SM has formed an adduct, PARP-1 is recruited and serves as a biological platform that utilizes nicotine adenine dinucleotide (NAD+) as a substrate to covalently bond poly-ADP- ribose (PAR) and other proteins to PARP-1 itself (known as automodification)[114,116]. Excision repair by PARP-1 in mild SM-DNA adduct formation will preserve the cellular integrity and viability and hinder mutations from occurring within the DNA chain[114,116]. Issues arise when the body is exposed to high or prolonged amounts of SM, leading to necrotic cellular damage[117,118]. The overwhelming demand for PARP-1 will lead to rapid NAD+ and adenosine triphosphate (ATP) depletion causing cellular rupture[113,114,119]. Depleting the NAD+ pools in the cell will lead to a deficiency in ATP, critical to cellular energetics and ultimately the cell's fate[120]. Severe ATP deficiency will result in PARP not being cleaved by caspase 3, thus potentially resulting in necrosis (Figure 2)[121]. It is thought that PARP has a regulatory function if it may need to shift from apoptosis to necrosis[121,122]. SM does cause necrosis as well as apoptosis at the site of injury via the Fas/FasL pathway[123]. When this pathway is activated, PARP is cleaved by caspase 3, and cells undergo apoptosis (Figure 2)[123]. The cell's inability to produce or use energy for vital functions for its survival leads to cell death, either via apoptosis or necrosis, resulting in extensive tissue damage. Extensive cell death leads to immune activation and the formation of oxidative stress.

Inflammatory cell activation: The inflammatory process is complex. Many pro-inflammatory and anti-inflammatory mediators play a critical role in the various inflammatory mechanisms that initiate inflammation and repair at the injury site. When SM is inhaled, it's been shown that significant necrosis occurs amongst epithelial cells and,

in turn, releases various mediators such as IL-1α to activate immune cells to the site of injury[124–126]. Aside from necrosis, ciliated cells become damaged and no longer have cilia which become critical in mucus clearance (Figure 2)[127]. In *in vitro* experiments, it has been shown that SM increases the production Muc5Ac increasing mucus[128]. When the epithelial cells become damaged, cellular matrix adhesion is lost, and the membrane becomes leaky (Figure 2)[128]. Aside from resident tissue immune cells such as mast cells and macrophages, the leaky membrane and pro-inflammatory mediators released from the necrotic cells and resident inflammatory cells signal more inflammatory cells to the site of injury. In the bronchioalveolar lavage (BAL) rodent models, there is an increase in IL-6 and TNFα, which has been attributed to macrophages (Figure 2)[129,130].

Furthermore, in the BAL, patients who exhibited SM-induced pulmonary fibrosis had elevated macrophage inflammatory protein 1α (MIP-1α) and monocyte chemoattractant protein −1 (MCP-1, CCL-2)[131]. The release of MIP-1α is known to attract neutrophils to the site of injury (Figure 2)[131]. Various animal models depict the accumulation of neutrophils following inhaled SM. Neutrophils are known to accumulate within minutes to hours of SM exposure[132]. Macrophages release various proinflammatory cytokines such as IL-1β, TNFα, IL-6, and MIP1α to recruit neutrophils to the site of injury[129,133]. Once at the affected tissue, neutrophils also release pro-inflammatory cytokines such as IL-1β, TNFα, IL-6, and IL-8[132,134].

Oxidative Stress Generation and Cellular Response: As the internal cellular homeostasis is disturbed by DNA damage, overactivation of PARP-1, and reduction of the NAD+ and ATP cellular pools, the cells located at the site of injury form an abundance of reactive oxygen species (ROS)[135]. A sign of early toxicity is inflammation, which can be triggered in response to ROS formation directly/indirectly from cell death and resident tissue cells signaling the initiation of an immune response. After inhalation of SM, macrophages neutrophils become a major source of ROS and nitric oxide (NO) production at the site of injury[136,137]. It has been hypothesized that these immune effector cells can produce ROS in what is known as a respiratory burst or oxidative burst[138–140]. As the name implies, these cells can rapidly release ROS to mediate the damage and initiate repair[140]. However, if the excess ROS is not scavenged, it causes oxidative stress to neighbor cells in the affected region, resulting in more damage before repair, including mitochondrial dysfunction[140,141]. While macrophages and neutrophils remain primary extracellular sources for NO and ROS, mitochondria within epithelial cells represent intracellular sources of oxidative stress[122]. Mitochondrial dysfunction leads to the reduction of detoxifying thiols such as glutathione (GSH)[142,143]. The decrease in GSH paired with the overproduction of ROS will lead to an imbalance in redox factors and changes in cellular metabolism[142,144]. These changes directly impact DNA base oxidation, lipid peroxidation, and protein oxidation[113,142]. As oxidative stress lingers, extensive damage to the cell membrane also occurs and contributes to secondary damage induced by SM, leading to such conditions as bronchiolitis obliterans, asthma, and chronic obstructive pulmonary disease (COPD). Overall, ROS is one of the major causes of chronic toxicity to SM exposure. Much debate remains around whether ROS initiates inflammation and mitochondrial dysfunction or does DNA alkylation events and damage trigger an inflammatory response, leading to ROS increase.

Inhibition of NADPH-cytochrome P450 reductase: Another mechanism that contributes to SM toxicity is the inhibition of NADPH-cytochrome P450 reductase[145]. Cytochrome P450 are enzymes that are abundantly found in the liver but are also located in many extrahepatic cells, including type II epithelial cells in the lung[146]. Additionally, the enzyme NADPH- cytochrome P450 reductase undergoes oxidation to donate an electron to cytochrome P450 to detoxify SM and its metabolites[145]. While this mechanism isn't as extensively studied as previously mentioned, it has been shown in the literature that SM is a potent inhibitor of cytochrome P450 and NADPH-cytochrome P450 reductase[145,147]. If SM exposure is persistent and overloads the body, the inhibition of NADPH-cytochrome P450 reductase cannot detoxify SM and its metabolites. The excess of SM and its metabolites are readily available to react with other biomolecules.

Since many of the previously mentioned mechanisms have been individually studied based on the effects of SM, there is a clear connection of how each mechanism plays a critical role within each other. What remains unclear is what mechanism is initiated first, and how SM primarily exerts its toxicity. Once this is determined, a viable treatment option can be developed.

Respiratory Effects After Sulfur Mustard Inhalation

Veterans and civilians exposed to acute low dose SM will experience symptoms within 12-24 h such as rhinitis, sneezing, bloody nose, burning sensation of the nose and throat, shortness of breath, and a dry cough[148]. Those who have been exposed to a much higher concentrations experience these symptoms within 2-4 h according to the CDC (LCt_{50} = 3000mg-min/m^3)[35,148]. Long-term effects from a one-time single exposure result in dyspnea, bronchitis, asthma, pulmonary fibrosis, bronchiolitis obliterans, and COPD[148,149]. While the mortality rate is low, most veterans experience chronic toxicity originating from the lung, attributed to the robust immune response[35]. This immune response is thought to impact many other organ systems further negatively throughout the body years after the exposure[150].

While respiratory effects are less studied than effects in the skin and the eyes, it is thought that inflammatory processes occurring in the lung play a major role in long-term disease. One of the most studied cohorts are veterans who served in the Iraq-Iran war (1986). This cohort had known direct SM exposures while veterans who served in the GW and Iraq-Afghanistan war were thought to be exposed to a mixture of chemical warfare agents. The in-depth analysis of the Iran-Iraq cohort has allowed us to identify similarities between the veterans who have served in the GW and the Iraq-Afghanistan war. Veterans from all three wars have reported similar disease pathologies such as increased asthma, COPD, difficulty breathing, and bronchiolitis obliterans, years after exposure[30,151,152]. Several studies regarding the Iranian cohort of veterans determined that 10 to 20 years after SM exposures, the most affected organ is the lung (42.5-95%) compared to the skin and eyes[153,154]. In further assessing lung damage years after exposure in Iranian veterans, chest X-ray findings and spirometry tests were used.

Findings confirm approximately 5% of veterans have increased pleural thickening, 41% have increased vascular markings, and 37.3% have pathology consistent with

bronchiectasis[155]. The spirometry test revealed obstructive breathing patterns in half of the veterans (110/220 cases)[155]. Moreover, studies have looked at inflammatory markers in exposed Iranian veterans' BAL fluid. Analysis of the BAL fluid revealed a significant elevation in various chemokines such as MCP-1, MIP-1α, MIP-1β together with elevated neutrophils, lymphocytes, and eosinophils compared to control initial exposure in 1986, confirming the presence of persistent inflammation[131]. Similarly, there have been reports of elevated proinflammatory cytokines such as IL-6, TNF-α, and IL-1β in the same cohort of veterans[131,134].. There has been a notable increase in bronchiolitis obliterans and evidence of pulmonary fibrosis amongst other respiratory issues such as chronic pulmonary obstruction, chronic bronchitis, and acute respiratory distress syndrome (ARDS)[15,151,156]. A recent study looked at the 10-year prevalence rate for fifteen respiratory diseases affecting veterans who actively served in the GW and veterans who were not deployed. Out of the 15 respiratory illnesses that were considered, this study found that per 100,000 veterans; there was an increase in the prevalence of chronic bronchitis (PR 1.19; 95% CI 1.10, 1.28), emphysema (PR 1.11; 95% CI 1.01, 1.21), chronic airway obstruction (PR 1.09; 95% CI 1.07, 1.12), and chronic obstructive pulmonary disease (PR 1.09; 1.07, 1.11)[151]. Many of these respiratory illnesses in this study overlap with the respiratory diseases experienced by Iranian veterans. While the literature fails to link GW veterans to SM-only exposures due to the many other exposures that occurred during the GW, it is essential to note that the similar lung pathology and respiratory symptoms experienced by Iranian veterans exposed to SM are analogous to the respiratory symptoms that comprise GWI. In understanding the mechanism of action of SM, there have been many advances in *in vitro* and *in vivo* research supporting the respiratory pathology in GWI.

Rat models have been extensively used in looking at the damage and acute inflammatory responses within the lung. Rats exposed to a high dose of 2 mg/kg of SM via intratracheal instillation had ulcerations, shedding of the epithelium, pulmonary fibrosis, airway remodeling, and inflammatory cell infiltration within 6 h persisting at 72 h[157]. The results described are comparable to what soldiers and civilians have experienced after a high exposure. In characterizing lung inflammation in rats following SM exposure, there was an increase in T and B lymphocytes, increased macrophages, and decreased glutathione peroxidase (GPx) by 72 h in the BAL[157]. The inflammatory cell infiltration at 6 h of SM exposure combined with an increase in GPx further strengthens the hypothesis that oxidative stress and immune response contribute to lung damage. SM exposure is known to cause pulmonary fibrosis and bronchiolitis obliterans in humans exposed, as previously mentioned. A study looking at rats exposed to 1.0 mg/kg of SM further demonstrated that bronchiolitis obliterans and pulmonary fibrosis gradually intensified while lung function decreased[158]. By 28 days, TGF-β, platelet derived growth factor (PDGF), and plasminogen activator inhibitor type 1 (PAI-1) elevated in the BAL, suggesting airway remodeling and activation of pro-fibrotic pathways contributing to the collagen formation is observed[158]. While rodent models are typical when assessing the toxicity of SM, swine models have also been used. In female swine exposed to 60 (sublethal SM dose) and 100 μg/kg (LD_{40}) doses for 10 min showed an increase in damage measured by lung pathology scoring as well as various transcriptional changes[159]. This study utilized canonical pathway analysis and saw changes

in various pathways such as aryl hydrocarbon receptor signaling, Nrf-2 mediated oxidative stress, differences in immune response, and effects on cell signaling metabolism[159].

In studying SM exposure, a few surrogates are often employed due to SM's restricted use and toxic nature. The two most used surrogates that elicit comparable immune response and damage in the lung are 2-chloroethyl ethyl sulfide (CEES, half mustard) and mechlorethamine (HN2, Nitrogen Mustard; NM). For most *in vivo* studies using CEES, the liquid is aerosolized at concentrations of 7.5-10% CEES in 90-92.5% ethanol and delivered via a nose-only system[160,161]. After 12- 18 h of CEES exposure in rats, there was evidence of epithelial damage due to increases in tissue factor activity in BAL, decreases in microRNA −140-5p (a marker for acute lung injury in tissue), and an increase in superoxide dismutase (SOD)[162]. Paired with the damage observed, there was an increase in proinflammatory cytokines IL-6, TNF-α, and IL-1α/β[162]. The comparable inflammatory responses of SM and CEES that occur as early as 12 h post-exposure raises the question of whether the inflammatory processes are initiating damage or if the damage caused is causing the inflammatory responses.

NM has been extensively used in rat and mice models via intratracheal instillation and oropharyngeal aspiration to determine if inflammation is responsible for generating the bulk of the damage in the lung. NM exposure is associated with increases in proinflammatory cytokines such as IL-6, TNF-α, IL-1β, and growth factors (TGF-β) at doses 0.08 mg/kg – 0.25 mg/kg[133,163]. First responding cells of the immune system are commonly macrophages and mast cells. Both cells are believed to play a critical role in initiating inflammation[163–165]. From 12- 72 h after NM treatment, macrophages [M1 (cytotoxic) and M2 (pro-resolving)] at the site of injury generate oxidative stress, eicosanoid production, and cytokines (TNF-α and IL6) as well as chemokines, all contributing to the altered lung function in rats and mice[133,163]. In understanding the attenuation of lung injury in rats by using an anti-TNF-α antibody (15 mg/kg TNF-α antibody 30 min after initial intratracheal instillation and then every nine days for 28 days), there was a decrease in M1 macrophage, but M2 remained mostly unchanged[165]. In this study, TGF-β, a profibrotic mediator, was also significantly reduced[165]. In looking at such exposures in mice, 14 days after 0.08 mg/kg of NM delivered via oropharyngeal aspirations, there was an increased expression of high mobility group protein 1 (HMGB1) and TNF-α associated with macrophage accumulation in tissue as well as high numbers of M1 macrophages correlating with TGF-β and fibrosis formation[163].

In addition to macrophages, mast cells have very similar roles in the initiation and resolution of inflammatory responses due to NM exposure[164]. Mast cells are critical in the formation of fibrosis and are known for rapidly releasing granules with preformed mediators recruiting macrophages and neutrophils to the site of injury[166]. Within 72 h, SM exposure (0.2-0.6 mg/kg) correlates with an increase in TNF-α followed by infiltration of macrophages and neutrophils following a 3-day exposure[129]. This increase in macrophages and neutrophils three days after initial exposure may be due to the various mediators that mast cells release. In a recent study looking at the role of mast cells in C57BL/6J mice (Wildtype; WT) versus mast cell-deficient mice ($Kit^{w\text{-}sh}$) 3 days after NM exposure (0.125 mg/kg via oropharyngeal aspiration), there was significant damage and immune cell infiltration in the WT that was

absent in the mast cell deficient mice[164]. Additionally, there were increases in mRNA gene expression for proinflammatory mediators IL-6 and TNF-α in WT that were absent in mast cell deficient mice as well as the increase in eicosanoid formation (PGD_2) in WT compared to the mast cell deficient mice[164]. Furthermore, it's been reported while inflammatory responses are robust early on, 10 days and 30 days after initial exposure (0.625 and 0.312 mg/kg NM) in mice revealed an upregulation in pro-fibrotic biomarkers such as serine/threonine – selective protein kinases (p-ERK) and heat shock protein 90 in addition to an increase in collagen expression[167]. Mast cells play a prominent role in fibrosis and could be contributing to the collagen formation by producing TGF-β which is responsible for fibroblast proliferation and migration[168]. In identifying treatment targets, stabilizing mast cells could be a viable option for treatment immediately after SM exposure. While further research is needed to determine if mast cells or macrophages are viable treatment targets to reduce inflammation in the lung, inhalation to SM has also been implicated in damage in the brain[169–171].

Inhalation of Sulfur Mustard Impacts the Central Nervous System

Inhalation of SM also results in neurological symptoms such as impaired cognition, severe headaches, lethargy, sleep disturbances, and increased depression [172,173]. While SM-induced damage is known to target organs such as the skin, eyes, and the lung, the mechanism(s) by which SM exposure results in cognitive impairment has not been fully elucidated. In Iranian veterans, SM mustard exposure contributes to the breakdown of the blood-brain barrier (BBB), abnormalities in the cerebellum and hypothalamus, and the many microbleeds in areas where necrosis is present throughout the brain contributing to cognitive dysfunction [174]. Due to its lipophilic properties, the accumulation of SM in the brain of post-mortem veterans gives us reason to believe that SM toxicity impacts regions of the brain responsible for cognition[171]. Interestingly, cognitive deficits are prominent features associated with GWI and include cognitive and memory problems, mood dysfunction, sleep disorders, and chronic fatigue [80]. In addition to sharing similar cognitive deficits as seen in exposure to SM, GWI primarily affects those veterans deployed in wars mentioned previously where SM exposure has been documented [175].

Few studies have looked at the inhalation effects of SM on the brain, but it has been reported that significant neuronal and structural changes occur. Tekiner et al. exposed rats to a toxic dose of vaporized NM for 10 min (800 mg/m^3/min), resulting in significant vascular oedema around major blood vessels and capillaries, abnormalities in neuronal and glial processes, and substantial changes in cellular components such as evidence of nuclear and mitochondrial degeneration[176]. Further analyzing whole brain tissue homogenates showed a significant increase in thiobarbituric acid reactive substances (TBARS), SOD activity, and GPx activity after NM exposure[176]. The same group wanted to determine if introducing the antioxidant proanthocyanin (PC) in the feed (100 mg/kg/day) would inhibit effects caused by NM exposure[176]. Their findings confirmed that the administration of an antioxidant such as PC could alleviate oxidative stress and lipid peroxidation in various brain regions and inhibit cellular degeneration[176]. Other studies have looked at the effects of SM, NM, and CEES on the brain through different modes of exposure, such as intraperitoneal (*i.p.*)

and diffusion through the skin. Although the routes of exposure are different, there are comparable results of neuronal degeneration and ROS formation in the brain.

Potential Therapeutics and Therapeutic Targets

Considering there are no therapeutic options, much of the current research focuses on identifying potential targets to develop therapeutics to treat individuals exposed through inhalation of SM. Many anti-inflammatory drugs have been used in vivo. In acute studies involving inhalation exposure to SM, NM, or CEES, they have shown promises, such as macrolides, glucocorticoid steroids, antioxidants, and anti-TNF-α antibodies. Macrolides are antibiotics typically used in the treatment of bacterial infections. In rats exposed to 0.25 mg of SM delivered via an intratracheal tube, roxithromycin (commonly used macrolide) given 1 h prior to exposure and therein 24 h after for 1, 3, and 7 days found that at three days there was a significant improvement in lung function[177].

Additionally, lung scores and survival at all doses of roxithromycin (10, 20, 40 mg/kg) improved in a dose-dependent manner[177]. Glucocorticoid steroids such as betamethasone and dexamethasone have been around for a few decades and have been previously used in guinea pigs exposed to SM[178,179]. Administration of both glucocorticoid steroids decreased airway hyperresponsiveness and repaired parts of the airway epithelium in SM exposed guinea pigs[179]. In addition to this study, C57BL/6J mice were exposed to 10 mg/kg of NM, and dexamethasone was administered (*i.p.*) once after either 1, 2, or 6 h post-exposure[180]. Dexamethasone protected the airways of mice significantly at all time points through the reduction of inflammatory cell infiltration[180].

Interestingly at 1 h of dexamethasone administration, there was better inhibition of collagen formation after 14 days[180]. Early treatment with glucocorticoid steroids in both SM and NM models (guinea pigs and mice) shows a significant reduction in inflammatory cells at different concentrations, making it a viable prophylactic[179,180]. As previously mentioned, ROS is a substantial contributor to injury. Metalloporphyrin catalytic antioxidant AEOL 10150 (after 1 and 9 h of 5mg/kg *s.c.*) showed significant improvement in the lungs of mice that were exposed to 5% of CEES for 15 minutes[181]. Oxidative stress markers 8-OHdG and 4-HNE were significantly decreased in the lung, which demonstrates AEOL10150 is an efficient antioxidant agent[181]. Other studies have shown the elevation of TNF-α after NM or SM inhalation exposures in rodent models. Treatment with anti-TNF-α was associated with a reduction in collagen deposition and suppression of proinflammatory cytotoxic macrophages in NM exposed lung[165]. Another therapeutics target being considered is an extracellular nucleic acid (eNA), which are essential mediators in the inflammatory process and damage[161,182]. With eNA's being a potential therapeutic target, this study utilizes eNA scavenger hexadimethrine bromide[161]. Twelve hours after a 10% CEES inhalation exposure in rats, there was an increase in eNA in the BAL of the lungs[161]. Upregulation in eNA in the BAL can lead to inflammation and barrier dysfunction within the lungs[161]. Hexadimethrine bromide is an extracellular nucleic acid scavenger[161,183]. Hexadimethrine bromide (10 mg/kg) was administered to rats 2 h post-exposure and resulted in improved blood oxygenation, reversal of ARDS, and little to no alveolar-capillary barrier damage[161]. While there aren't any approved treatment options to prevent the pathophysiological effects

of SM exposure, various cellular targets have been identified for potential therapeutics. There is a clear need to identify therapeutics further potentially targeting vital immune cells such as mast cells and macrophages or oxidative stress based on our knowledge of mechanisms of toxicity.

Iraq and Afghanistan Wars

Many of the exposures experienced in the GW were also experienced in the Iraq-Afghanistan war but over a longer time frame. Soldiers during the Iraq War were exposed to aging chemical weapons left over from the Iran-Iraq War[36]. Thus far, in the Iraq war, there were reports of CWA exposure in both operations Iraqi Freedom and New Dawn[36]. Little is known about the chemical exposure experienced during the Afghanistan war since it ended very recently in August 2021. Although many veterans' self-reported exposures to GB, there is difficulty in corroborating the reports with military records[28]. This lack of corroboration is a significant issue as researchers cannot confirm that soldiers self-reporting exposures to chemical weapons were actually near any confirmed sites of chemical attack or weapons storage and/or demolition[28]. In one study using veteran self-reports, many veterans reported receiving PB, even though they were not supposed to have been administered PB [28]. In recent military operations, there have been reports of soldiers being exposed to SM through the handling and destruction of explosives during the Iraq- Afghanistan conflicts between 2003 to 2015[28]. While this information has been reported in the media, the exact number of U.S. soldiers that were exposed remains unconfirmed.

As a result, veterans who served in the Iraq-Afghanistan war (2001-2021) are experiencing similar symptoms[27–29]. Amidst the 2.5 million men and women deployed to the Iraq-Afghanistan war, also known as the "War on Terror" (post 9/11/2001 terrorist attacks), many veterans who have returned experienced symptoms related to CMI. One year after returning from deployment, 49.5% of veterans met the criteria for CMI, similar to that seen in GW veterans[27]. Respiratory issues were one of the many reported symptoms expressed by veterans[27,30]. There was a 28% increase in veterans experiencing allergies and a 19% increase in asthma after soldiers returned from deployment[30]. To further assess military exposure and long-term health outcomes, the Millennium cohort group was established in 2001. This prospective study conducted by the DoD reported an increase in mental and respiratory illness months after arriving from deployment[27]. Veterans in the Millennium cohort had trouble sleeping (51.4%), were moody/irritable (50.8%), had joint pain (46.0%), fatigue (39.5%), difficulty remembering (39.8%), concentration issues, headaches (36.1%), and sinus problems (30.0%)[27]. Veterans who served in the GW and Iraq- Afghanistan war have reported lung disease pathologies similar to those in SM-exposed veterans of the Iran-Iraq War.

Due to the nature of the recently concluded conflict in Afghanistan (2021) and the exposures troops experienced that parallel those in the GW, there is more to be learned on the factors potentially contributing to the CMI observed in the Iraq-Afghanistan veterans.

Conclusion

Over 30% of veterans who served in the Persian Gulf War have suffered respiratory and cognitive effects due to various exposures, including CWAs[35]. While there isn't an exact number of veterans affected from the Afghanistan- Iraq war that recently ended (2021), there is a rising number of reports of respiratory and cognitive effects[28].

With GWI/CMI recognized in both veteran populations, and exposures to CWAs occurring during both wars, it is suggested that CWAs such as GB, GF, and SM have critical roles in the manifestation of this illness[5,29,46,75]. While there are few reports of the lungs being affected in GB and GF exposures, the mode by which many of these animal models are exposed is not what is typically experienced during the war (*i.p.* or *s.c.*) when studying respiratory effects. Due to GB and GF being an OP compound, the vast knowledge in the literature revolves around neurological effects, and very little explores the respiratory damage experienced by veterans as a result of inhaling these compounds. This needs to be addressed because pulmonary injury and inflammation are critical factors in GWI/CMI[15,30]. The known neurocognitive effects resulting from GB/GF exposure are thought to involve hippocampal injury, neuroinflammation, and AChE inhibition[74,75]. It remains unclear what mechanisms contribute to neuroinflammation and hippocampal injury and why veterans experience memory loss years after exposure[74,75]. Long-term animal studies could help bridge this gap in the literature. Although GB, GF, and SM are highly toxic chemicals, many veterans exposed do not die from the exposure itself, rather experience various symptoms years after exposure[35,64,75,148]. Long-term animal studies will help further identify mechanisms and biomarkers that may contribute to both lung and brain pathology encountered in veterans.

Sulfur mustard is also believed to play a critical role in the manifestation of GWI. Many of the symptoms manifested in GWI have also been observed in Iranian veterans from the Iran-Iraq war who had known SM exposures[150,151,174]. While surrogates are valuable in understanding the potential mechanisms that SM is implicated in, many of these studies need to be carried out further using the actual compound SM. It has been shown that CEES and NM compared to SM have similar immune effects and damage on the lung and various other organs, but there are still differences in toxicity amongst these compounds[184–186]. In addition to using the actual compound SM, the literature lacks exploring the neurocognitive effects of SM exposure and its role in neuronal damage. There is evidence that the parent compound, SM, accumulates in post-mortem patients, and such exposure can contribute to cognition decline experienced by veterans[171,173,174]. These findings have been shown in Iranian veterans, but very few animal studies have addressed mechanisms.

Both wars previously mentioned had various other exposures such as depleted uranium (DU), pesticides (organophosphates and carbamates), fine particulate matter (PM) (building fires, oil well fires, and desert dust), and CWAs were not exclusive to either war. With what is already known about CWA's and the injury it has inflicted on various military personnel, it does appear that CWA's may play a critical role in the disease pathology seen in GWI/CMI.

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List of abbreviations:

ACh	Acetylcholine
AChE	Acetylcholinesterase
ALS	Amyotrophic lateral sclerosis
ARDS	Acute respiratory distress syndrome
ATP	Adenosine triphosphate
BAL	Bronchioalveolar lavage
BBB	Blood-brain barrier
BChE	Butyrylcholinesterase
CDC	Center for Disease Control
CEES	2-chloroethyl ethyl sulfide
CMI	Chronic multisymptom illness
CNS	Central nervous system
COPD	Chronic obstructive pulmonary disease
CPO	Chlorpyrifos-oxon
CWA	Chemical warfare agent
DFP	Diisopropyl fluorophosphate
DFPase	Diisopropyl fluorophosphatase
DoD	Department of Defense
DU	Depleted uranium
eNA	extracellular nucleic acid
FDC	Ft. Devens cohort
GB	Sarin
GF	Cyclosarin
GPx	Glutathione peroxidase
GSH	Glutathione

GW	Gulf war
GWECB	Gulf war era cohort and biorepository
GWI	Gulf war illness
HMGB1	High mobility group box 1 protein
MCP-1	Monocyte chemoattractant protein-1
MIP-1α	Macrophage inflammatory protein 1
NAD	Nicotine adenine dinucleotide
NHANES	National Health and Nutrition Examination Survey
NM	Nitrogen mustard
NO	Nitric oxide
OP	Organophosphate
PAI-1	Plasminogen activator inhibitor type 1
PAR	Poly-ADP-ribose
PARP-1	Poly (ADP-ribose) polymerase type1
PB	Pyridostigmine bromide
PC	Proanthocyanin
PD	Parkinson’s disease
PDGF	Platelet derived growth factor
PEG	Polyethylene glycol
PM	Particulate matter
PON1	Paraoxonase 1
ROS	Reactive oxygen species
SE	Status epilepticus
SM	Sulfur mustard
SOD	Superoxide dismutase
TBARS	Thiobarbituric acid reactive substances
VA	Veteran affairs

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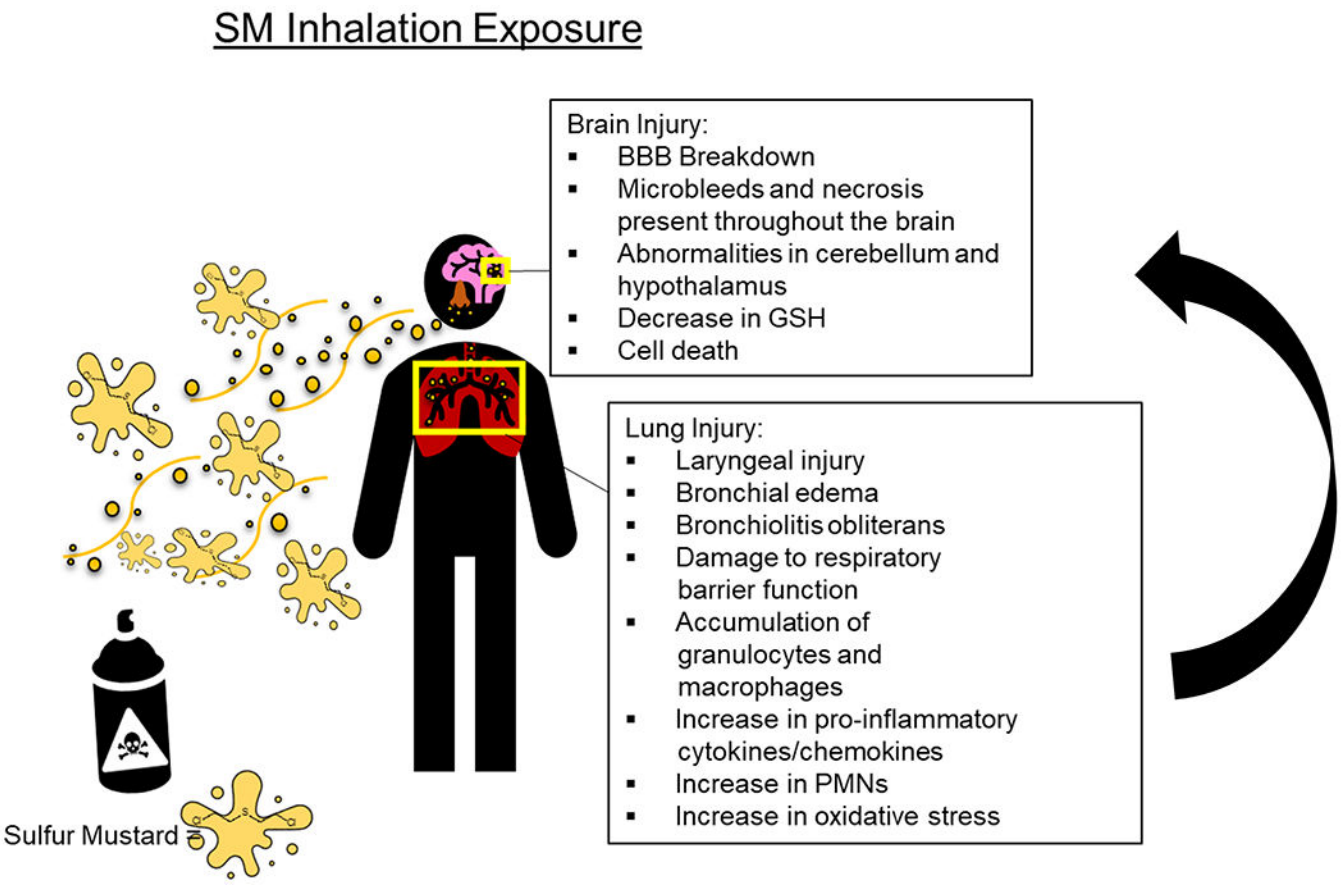

Figure 1. Overview of SM exposure.

Inhalation to SM has been attributed to lung and brain injury. After exposure to SM, soldiers and civilians develop respiratory complications, such as asthma-like pathologies, chronic bronchitis, and pulmonary fibrosis. While the lungs are known for their susceptibility to SM toxicity, the brain's effects remain primarily unknown. Figure created with biorender.com

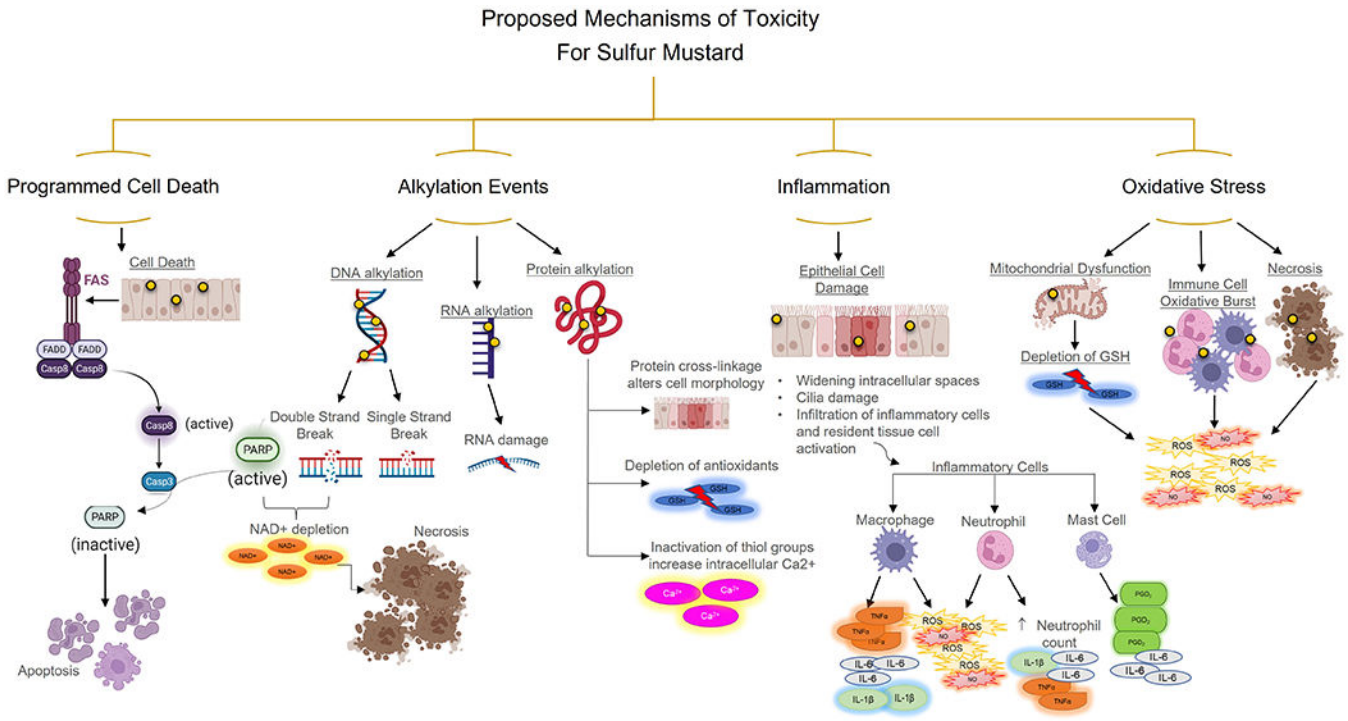

Figure 2. Overview of Proposed Mechanisms of Toxicity for Sulfur Mustard.

(A) SM can cause apoptosis via the FAS/FASL pathway. (B) SM alkylates DNA, RNA, and proteins. DNA alkylation leads to single and double-strand breaks, which leads to the activation of PARP. Overactivation of PARP leads to NAD^+ depletion and necrosis. While RNA and protein alkylation don't lead directly to cell death, lack of antioxidants increases intracellular Ca^{2+} and alters cell morphology. (C) SM's damage and toxicity causes epithelial damage leading to a robust inflammatory response through macrophages, neutrophils, and mast cells. (D) SM causes mitochondrial dysfunction, immune cell accumulation (macrophage and neutrophils), leading to a respiratory burst of reactive oxygen species (ROS), nitric oxide (NO), and necrotic cell death. Figure created with biorender.com

Table 1:

Studies on the toxic effect of pyridostigmine bromide.

Pyridostigmine Bromide			
Study (Ref #)	**Model**	**Exposure**	**Results**
Bryant et al., 2021 (23)	Male and female C57BL/6 mice (WT, NLRP3$^{-/-}$, and STING$^{gt/gt}$)	0.7 mg/kg PB and 200 mg/kg Per in 50 mL DMSO daily for 10 days	Greater neuroinflammation and cognitive impairment in female mice; greater peripheral inflammation in males
Hernandez et al., 2020 (25)	Male and female C57BL/6 mice	90 mg/mL PB in drinking water for 7 days; 0.07 mg/mL PEA in drinking water for one month	PB alone increased fecal pellet production and colonic transit time only in females, PB+PEA increased myenteric neurons and muscarinic M3 receptors only in females
Hernandez et al., 2019 (26)	Male and female C57BL/6 mice	9 or 90 mg/mL PB in drinking water for 7 days	PB-induced neuroimmune effects seen in males immediately after treatment, but not in females until 30 days after beginning of treatment

Table 2:

Studies on the toxic effect of organophosphates.

Organophosphates			
Study (Ref #)	**Model**	**Exposure**	**Results**
Maxwell et al., 2013 (40)	Recombinant human AChE	AChE incubated with OP compounds, including sarin and tabun	OP compounds deactivated AChE
Terry et al., 2011 (49)	Male albino Wistar rats	0.25, 0.5, 0.75, or 1.0 mg/kg DFP, CAS 55-91-4 every other day for 30 days	DFP could cause cognitive impairment in doses that do not cause cholinergic distress
Locker et al., 2017 (50)	Male C57BL/6 mice	8 mg/kg CPO, 4 mg/kg DFP, 3 mg/kg PB, or 0.5 mg/kg PHY; 400 mg/L CORT in drinking water for 4 days before treatment with AChE inhibitor	Only OP AChE inhibitors cause neuroinflammation; stress increases neuroinflammation and decreases AChE inhibition; Importance of AChE in OP-induced neuroinflammation rejected
Miller at al., 2018 (51)	Male C57BL/6 mice	8 mg/kg CPO, 4 mg/kg DFP, 3 mg/kg PB, or 0.5 mg/kg PHY; 400 mg/L CORT in drinking water for 4 days before treatment with AChE inhibitor	Stress exacerbates OP-induced neuroinflammation; Markers of neuroinflammation do not correlate with AChE activity in the brain
O'Callaghan et al., 2015 (52)	Male C57BL/6 mice	2 mg/kg PB and 30 mg/kg DEET daily on days 1-14 (100 mg/kg MINO on days 1-15 for MINO experiment); 200 mg/L CORT in drinking water on days 8-15, 4 mg/kg DFP on day 15	CORT exacerbated DFP-induced neuroinflammation and removed protective effects of PB/DEET; MINO suppressed CORT+DFP-induced neuroinflammation; Organophosphorylation implicated in neuroinflammation
Yates et al., 2021 (53)	hiPSC cells generated from PBMCs from GW veterans; male albino Wistar rats	Cells exposed to 2 mM cortisol and either 200 nM, 1 mM, or 2.5 mM DFP; Rats exposed to 0.4 mg/kg CORT in drinking water for 7 days and then given 1.5 mg/kg DFP on day 7	Altered tau levels, microtubule acetylation, and mitochondrial function in hiPSC cells; increased total tau, decreased total cell numbers,but unaltered cell morphology in CA3 region of hippocampus
Lein, P.J. & Fryer, A.D., 2005 (54)	Male guinea pigs	0.1-10 mg/kg parathion, 0.75-75 mg/kg diazinon, or 150 mg/kg permethrin	Exacerbated vagally induced bronchoconstriction and bradycardia without causing cholinergic distress; no exacerbation of Ach-induced bronchoconstriction
Katz, L.S. & Marquis, J.K., 1989 (55)	Caudate nuclei harvested from calf brains	Displacement of [^{3}H]QNB by muscarinic antagonists was measured in the presence or absence of paraoxon (dose not given)	Paraoxon, an OP compound inhibits [^{3}H]QNB
Guignet et al., 2020 (57)	Male Sprague Dawley rats	0.1 mg/kg PB and either 4 mg/kg DFP or vehicle	Reactive astrocytes, microgliosis, and increased oxidative stress in brains of OP-treated rats
Zhang et al., 2021 (59)	Male C57BL/6 mice	0.01, 0.1, 1, or 10 mg/kg CPF for five days per week for 12 weeks	Liver inflammation and altered gut microbiota
Meng et al., 2022 (60)	Female BALB/c mice	10 or 100 mg/kg TnBP or 10 or 100 mg/kg TBOEP each day for three weeks	Lung inflammation and exacerbated allergic response
Pena-Philippides et al., 2007 (67)	Male Fischer 344 rats	10 mg/kg CHL 7 days before sarin exposure; 0.4 mg/m^3/h/day sarin for 1 or 5 days	Significantly increased gene expression of TNF-a, IL-1b, and IL-6; effects did not persist beyond two weeks
Pena-Philippides et al., 2007 (67)	Male Fischer 344 rats	10 mg/kg CHL 7 days before sarin exposure; 0.4 mg/m^3/h/day sarin for 1 or 5 days	Significantly increased gene expression of TNF-a, IL-1b, and IL-6; effects did not persist beyond two weeks
Kassa et al., 2004 (68)	Female BALB/c mice	Clinically asymptomatic concentration of sarin given either once or three times each other day via inhalation	Inhibition of T-cell function
Gundavarapu et al., 2014 (71)	Male F344 rats	13-15 mg/m^3 sarin for 10 min	Severe bronchoconstriction

Organophosphates			
Study (Ref #)	**Model**	**Exposure**	**Results**
Shi et al., 2021(76)	Male guinea pigs	16.8 mg/kg sarin daily for 14 days	Disrupted lipid metabolism in hippocampus
Genovese et al., 2006 (83)	Male Sprague-Dawley rats	16-5.2 mg/m^3 cyclosarin	Impaired learning; recovered months later
Phillips and Deshpande, 2016 (85)	Male Sprague-Dawley rats	400 mg/kg DFP daily for 5 days	Severe cognitive impairment and neuronal cell death in hippocampus three months after exposure
Valiyaveettil et al., 2011 (89)	Male Hartley guinea pigs	846 mg/m^3 sarin or 841 mg/m^3 soman; 1 mg purified human or 0.5 mg purified rabbit serum PON1	PON1 protected against sarin and soman
Raveh et al., 1993 (92)	Male albino ICR mice and male Sprague-Dawley rats	12-45 nmol HuBChE and various doses of OP compounds	HuBChE protects against OP compound-induced toxicity
Worek et al., 2014 (96)	Male Dunkin-Hartley guinea pigs	0.2 or 1.0 mg/kg IlG1 and 100 mg/kg cyclosarin	Prevented cyclosarin toxicity
Melzer et al., 2012 (97)	Male Wistar rats	270 mg/kg soman; 12.2, 35.8, or 71.0 mg/kg WT DFPase; 35.8 mg/kg PEGylated DFPase	High dose of DFPase prevented soman-induced mortality
Kuruba et al., 2018 (98)	Male Sprague-Dawley rats	3.2 mg/kg DFP; 5 mg/kg Diazepam at 10 minute, 60 minute, or 120 minutes post-DFP exposure	Diazepam effective when given at 10 minutes, but completely ineffective at later time points
Reddy et al., 2020 (99)	Male Sprague-Dawley rats	3.2 mg/kg DFP;30, 60, or 100 mg/kg phenobarbital	Highest dose of phenobarbital protected against DFP-induced seizures and neuronal damage, but could also cause coma or death
Rojas et al., 2018 (100)	Sprague-Dawley rats (sex unspecified)	9.5 mg/kg i.p. or 5 mg/kg s.c. DFP; 10 mg/kg diazepam; 0.8 g/kg urethane	Urethane ended DFP-induced seizures and prevented further seizures
Rojas et al., 2020 (101)	Male Sprague-Dawley rats	5 mg/kg DFP; 0.8 g/kg urethane	Urethane suppresses neurodegeneration and inflammatory mediators
Lumley et al., 2019 (102)	Male Sprague-Dawley rats	453 mg-min/m^3 of GB for 60 minutes; 10 mg/kg diazepam and either vehicle or 4 mg/kg pregnanolone	Diazepam plus pregnanolone reduced seizures and neurodegeneration
Zhu et al., 2020 (105)	Male Sprague-Dawley rats	0.5 mg/kg DFP daily for 5 days; various doses of ketamine, R-ketamine, or S-ketamine	Ketamine improved behavior

Table 3:

Studies on the toxic effect of vesicants (sulfur mustard, nitrogen mustard, and CEES).

Pyridostigmine Bromide			
Study (Ref #)	**Model**	**Exposure**	**Results**
Bryant et al., 2021 (23)	Male and female C57BL/6 mice (WT, NLRP3$^{-/-}$, and STING$^{gt/gt}$)	0.7 mg/kg PB and 200 mg/kg Per in 50 mL DMSO daily for 10 days	Greater neuroinflammation and cognitive impairment in female mice; greater peripheral inflammation in males
Hernandez et al., 2020 (25)	Male and female C57BL/6 mice	90 mg/mL PB in drinking water for 7 days; 0.07 mg/mL PEA in drinking water for one month	PB alone increased fecal pellet production and colonic transit time only in females, PB+PEA increased myenteric neurons and muscarinic M3 receptors only in females
Sulfur Mustard			
Study (Ref #)	**Model**	**Exposure**	**Results**
Inturi et al., 2014 (117)	JB6 cells	0.75 mM nitrogen mustard	Decreased cell growth; cell cycle arrest
Byers et al, 2000 (120)	Hairless guinea pigs	7 minutes exposure to 10 mL sulfur mustard in vapor cups	Sulfur mustard depletes NAD+, leading to ATP deficiency and cell death
Malaviya et al., 2010 (124)	Male Crl:CD (SD) rats	0.7, 1.0, or 1.4 mg/kg sulfur mustard via inhalation	Damage, cell death, and inflammation in the lungs
Chevillard et al., 1992 (127)	Respiratory epithelial (tracheae of male New Zealand white rabbits)	0.1 mM sulfur mustard	Cessation of ciliary beating
Malaviya et al., 2020 (129)	Male Wistar rats	0.06-0.6 mg/kg sulfur mustard via inhalation	Increased TNF-α in lungs
Feng et al., 2019 (130)	Male ICR mice	40 mg/kg SM for survival and respiratory function experiments; 30 mg/kg SM for other experiments	Increased pro-inflammatory cytokine expression in lungs
Sunil et al., 2011 (133)	Male Wistar rats	0.25 mg/kg nitrogen mustard	Alveolar macrophages increased in size and number; increased neutrophil numbers
Gray et al., 2010 (145)	MLE-15 murine lung epithelial cells	100 mg/mL CEES	NADPH cytochrome P450 reductase inhibited in CEES-treated cells
Xiaoji et al., 2016 (157)	Male Sprague-Dawley rats	2 mg/kg SM	Tissue damage and inflammatory cell infiltration in the lungs
McGraw et al., 2018 (158)	Male Sprague-Dawley rats	1.0 +/− 0.05 mg/kg SM	Decreased lung function; development of bronchiolitis obliterans and pulmonary fibrosis
Jugg et al., 2016 (159)	Female large white pigs	60 or 100 mg/kg SM	Lung damage and alterations in pathways such as the Nrf2 pathway
Rana et al., 2020 (162)	Male Sprague-Dawley rats	15 minutes exposure to CEES vapor	Epithelial damage, decreased microRNA-140-5p, increased pro-inflammatory cytokines, and increased superoxide dismutase
Sunil et al., 2020 (163)	Male and female C57BL6/J mice	0.8 mg/kg NM	Tissue damage and inflammation in the lung

Pyridostigmine Bromide			
Study (Ref #)	**Model**	**Exposure**	**Results**
Cruz-Hernandez et al., 2021 (164)	Male C57BL/6J and Kit$^{W\text{-}sh}$ mice; BMMCs from C57BL/6J mice	0.125 mg/kg NM	Inflammation and lung injury occurred in C57BL/6 mice, but not in the mast cell-deficient mice
Malaviya et al., 2015 (165)	Male Wistar rats	0.125 mg/kg NM; 15 mg/kg anti-TNFa antibody every nine days after NM exposure	Anti-TNFa antibody reduced M1 macrophage numbers and TGF-b levels
Solopov et al., 2020 (167)	Male C57BL/6J mice	0.312 or 0.625 mg/kg NM	Increased pro-fibrotic biomarkers and collagen expression at 10 and 30-days post-exposure
Tekiner et al., 2009 (176)	Male brown rats	800 mg/m^3/min NM for 10 minutes; 100 mg/kg/day proanthocyanin	Vascular edema, abnormal neuronal and glial processes, and altered cellular components in the brain; proanthocyanin alleviated NM-induced oxidative stress
Gao et al., 2011 (177)	Male Crl:CD SD BR rats	0.25 mg SM in 100 mL inhaled over 50 minutes; 10, 20, or 40 mg/kg roxithromycin	Roxithromycin improved lung function in SM-exposed rats
Calvet et al., 1994 (178)	Male Hartley guinea pigs	0.3 mL/kg SM injected into trachea; trachea incubated with 10^{-5} M phosphoramidon	Phosphoramidon inhibited SM-induced sensitivity to substance P
Calvet et al., 1996 (179)	Male Hartley guinea pigs	0.3 mL/kg SM (stored 100 mg/mL in EtOH and then diluted 1:100 in 0.9% NaCl) given intratracheally; 3 mg/kg betamethasone in drinking water for seven days	Betamethasone repaired airway damage caused by SM
Wigenstam et al., 2012 (180)	Female C57BL/6 mice	1 mg/kg melphalan; 10 mg/kg dexamethasone given 1, 2, or 6 hours after melphalan exposure	Dexamethasone reduced inflammatory cell infiltration in lungs of SM-exposed mice
O'Neill et al., 2010 (181)	Male Sprague-Dawley rats	5% CEES in ethanol at 12.7 cc/hour for 15 minutes; 5 mg/kg AEOL 10150 injected at 1 or 9-hours post-exposure	AEOL 10150 decreased markers of oxidative stress in SM-exposed rats
Sharma et al., 2010 (186)	Female Swiss mice	11.9 mg/kg HN-1, 20.0 mg/kg HN-2, 7.1 mg/kg HN-3, or 8.1 mg/kg SM	Differences in toxic effects and efficacy of antidotes seen between mustard compounds