SUMMARY

After the Persian Gulf War (1990–1991), many veterans reported such signs and symptoms as rashes, fatigue, muscle and joint pain, headaches, loss of memory, depression, abdominal pain and diarrhea, coughing, sneezing, choking sensations, chest pain, sleep disturbance, and hair loss.[1] Surveys indicate that roughly 4 percent developed their symptoms before the war, 25 percent during the war, and 25 percent in the year following the war. Nearly 50 percent developed their symptoms in the second and third years after the war—and beyond (Kroenke, 1998). This report reviews the scientific literature related to the health effects of one of the possible causes of these illnesses, chemical and biological warfare agents.[2] These are grouped as follows, with a chapter devoted to each group:

- skin damaging agents (mustards, lewisite, phosgene oxime)
- toxins (ricin, trichothecenes, aflatoxins)
- nerve agents (tabun, sarin, soman, cyclosarin, and VX).[3]

POSSIBLE EXPOSURE

Before the Gulf War, Iraq had made extensive use of chemical warfare against Iran and its own people (United Nations [UN], 1984; Cordesman and Wagner, 1990). It is also known that Iraq conducted a program of research and devel-

[1]Although the press has used the term "Gulf War Syndrome" to describe these illnesses, no single definition has been developed, and no new disease entity has been established (Gibbons 1998; Marshall, 1997; NIH 1994). This review uses the term Gulf War illnesses to refer to the health problems reported by Gulf War veterans.

[2]This report concentrates on chemical warfare agents and one class of biological warfare agents, called toxins. Toxins are produced by living organisms (microbes, fungi, plants, and animals), but it is actually the chemical they produce that is used, which makes these toxins different from other biological agents, such as anthrax, that kill as a result of their biological properties. Other biological warfare agents, anthrax and botulism, are reviewed in a separate RAND report on infectious diseases (Hilborne and Golomb, 2000).

[3]Little information was found on the chemical thiosarin.

opment of chemical and biological weapons. This capability was a major concern for coalition forces, which made great efforts to minimize the risk of exposure to chemical and biological warfare weapons by providing protective equipment, detectors, medications, and immunizations and by addressing the issue in troop training (Clancy and Franks, 1997).

During the air war (which began in mid-January 1991) and the short ground war (which ended in early March 1991), there were no obvious chemical or biological attacks on coalition forces. However, there were a number of alarms and ambiguous events involving chemical agents, and it is know that U.S. troops did blow up a large ammunition depot at Khamisiyah that contained chemical weapons. Many reports of detector alarms—which seem primarily to have stemmed from known interfering substances, such as smoke and engine exhausts—undoubtedly contributed to the perception of some veterans that they were exposed to chemical agents.

Coalition air attacks did strike some Iraqi chemical and biological facilities. However, studies modeling the release of nerve agents and mustards from such attacks indicate that significant transport of agent to the vicinity of U.S. forces was unlikely (Central Intelligence Agency [CIA], 1996). Some concern remains that low levels of exposure might have occurred from such attacks (U.S. General Accounting Office, 1997; Riegle and D'Amato, 1994).

We know of at least one chemical weapon exposure that involved U.S. forces, which took place shortly after the defeat of Iraqi forces. In the process of destroying an Iraqi weapon depot at Khamisiyah, U.S. forces blew up a bunker and stocks of rockets that contained the nerve agents sarin and cyclosarin without knowing that the rockets contained chemical warheads. No casualties occurred, and it was only much later determined that nerve agents were destroyed and probably released during the demolition. This event has been studied and modeled extensively. A number of models were developed to estimate exposures, which yielded estimates indicating that a large number of U.S. troops (perhaps as many as 100,000) were potentially exposed to very low levels of nerve agents—levels unlikely to cause any acute clinical symptoms.

A second incident has also been investigated extensively (OSAGWI, 1997d[4]). In that incident, a single U.S. soldier developed typical mustard agent–like blisters on his arm several hours after being in an Iraqi bunker while he was assisting in the destruction of Iraqi vehicles and military equipment. Whether the cause of the blisters was mustard agent or some other source is currently unresolved and continues to be investigated.

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[4]OSAGWI has made a large number of Gulf War–related documents available on line in addition to its own products For simplicity, all are listed under OSAGWI in the Bibliography.

As part of conditions imposed at the termination of hostilities, Iraqis were required to declare their holdings of chemicals and biological weapons and to permit UN inspectors to access facilities and oversee destruction of all chemical and biological weapons. As a result of UN Special Commission efforts, much more is known about Iraqi capabilities at the time of the war (Zilinskas, 1997). Still, the picture is far from complete, and the Iraqis have been far from candid.

APPROACH

This report describes the medical effects of the chemical and biological agents it was believed Iraq could have possessed. These agents are intended to kill or disable humans. Although we describe the effects of militarily effective higher-dose exposures, the primary focus of the paper is on the effects of lower-dose exposures, especially long-term or delayed effects. The information is intended to assist in the analysis of possible exposures, but this review does not attempt to determine if such exposures occurred or to determine their relationship to Gulf War illnesses. Such determinations are the responsibility of OSAGWI and others.

The literature did not yield either examples of clinical problems arising two or more years after agent exposure or mechanisms for such health developments. There were reviews of some longer-term medical problems that can arise from lower levels of exposure and of mechanisms that could persist for some time after exposure ceases.

PROBLEMS OF RECOGNITION

The ability of U.S. forces to recognize exposures, especially at low levels, depended on chemical detection and clinical recognition of the signs and symptoms of agent exposure. The review found that there might be situations in which health effects from exposures might not be recognized clinically.

U.S. detectors were unable to recognize toxins or biological agents. "Dusty" mustard (or "dusty" nerve agent) also would probably not have been recognized by CAM or M8A1 detectors because they act on an ion mobility principle (OSAGWI, 1990). Nerve agents would probably have been detected by monitors at concentrations below those that affect the eyes. This is certainly the case for tabun and sarin, but it is less certain for VX and other potent agents (Defense Science Board, 1994; NRC, 1997). These detection systems produced a large number of apparently false alarms (from smoke exhausts and other interfering chemicals), which perhaps caused some veterans to believe they had been exposed to agents.

Although exact agent identification would have been more difficult, clinical recognition of militarily efficient attacks would have been straightforward. The

recognition of lower-level exposures is more complex and uncertain for the following reasons:

- The signs and symptoms of lower-level exposures are not specific and can resemble other common health problems. Historically, victims may have misinterpreted many low-level exposures as common headaches, respiratory or gastrointestinal illness, asthma, or conjunctivitis (Gaon and Werne, 1955). As will be discussed with separate agents, there may be subtle points to help differentiate agent exposures from common problems. Table S.1 indicates the overlapping effects of the agents under review.
- Significant biological effects can occur from acute or cumulative exposures that do not produce obvious signs and symptoms (Gaon and Werne, 1955; Holmes, 1959; Bowers, Goodman, and Sim, 1964; Wolthuis, Groen, et al., 1995; Burchfiel, 1976; Stephens, Spurgeon, and Berry, 1996; Mayer, 1953a; Steyn, 1995). (Similar effects have been reported from structurally similar organophosphate pesticides.)
- Several of the agents do not have immediate effects following exposure. For example, effects from mustard exposure may not occur for several hours to several days (Wachtel, 1941; Vedder, 1925); ricin effects may not be apparent for hours to a day (Franz and Jaax, 1997); and even massive exposures to aflatoxin produce symptoms only after an 8- to 16-hour delay (Chao et al., 1991).
- Responses to agent exposure can vary substantially because of such factors as race, temperature, diet, gender, and time of day (Vedder, 1925). For example, circadian variations can produce changes such that exposure at one time of day may result in distinct illness, while an identical exposure 12 hours later there may be little sign from the same exposure (Elsmore, 1981).
- In the case of nerve agents, it is possible that prior administration of pyridostigmine bromide may decrease and shorten the clinical response (Gall, 1981; Husain, Kumar, Vijayaraghavan, et al., 1993; Vijayaraghavan et al., 1992). This has not been extensively studied at low dosages.
- There is also a condition of tolerance in which exposure to anticholinesterase chemicals results in decreased response to subsequent exposure to chemicals of the same class—so that exposures do not produce typical signs and symptoms. Single and repeated subclinical exposures produce tolerance (Costa, Schwab, and Murphy, 1982).

SKIN DAMAGING AGENTS

Lewisite, phosgene oxime, and mustard agents are all skin-damaging agents. Although they have some distinct chemical properties, they all affect the skin, eyes, and respiratory tract and, once absorbed, have systemic toxicity.

Table S.1

Signs and Symptoms of Agent Exposure

	Skin[a]	Eye[b]	Respiratory[c]	Gastrointestinal[d]	Musculoskeletal[e]	Systemic[f]	Mental[g]
Lewisite	X	X	X	X		X	
Phosgene-oxime	X	X	X				
Mustards	X	X	X	X		X	X
Ricin		X	X	X	X	X	
Trichothecenes	X	X	X	X		X	X
Aflatoxins		?	X	X	X	?	?
Nerve agents	X[h]	X	X	X	X	X	X

[a]Rash irritation, erythema, blisters, and itching.

[b]Irritation, tearing, redness, and blurred vision.

[c]Rhinorrhea, sore red throat, cough, tight chest, and wheezing.

[d]Nausea, vomiting, cramping, and diarrhea.

[e]Muscle cramps, aching joints, and muscles.

[f]Malaise, lethargy, and low fevers.

[g]Difficulty thinking or remembering, and sleep disorders.

[h]Local sweating, itching, and erythema.

Lewisite

Lewisite was developed during World War I and has not received much recent study. In high concentrations, this arsenic-containing agent produces irritation and blistering of the skin and injury to the eyes and lungs promptly after exposure. At lower levels, the effects resemble exposure to tear gas, with irritation of skin, eyes, and respiratory tract, which could be misinterpreted as being due to other irritants or infection. Chronically exposed munitions workers have developed chronic bronchitis, but arsenic-based neuropathy was not reported. Chronic exposure to lewisite may predispose to Bowen's squamous cell intraepithelial cancer of the skin. There is no indication that brief exposures to low levels of lewisite are associated with long-term problems. Lewisite degrades rapidly in the environment, making hazard from long-range transport unlikely. Some Fox vehicles reported detecting lewisite during the Gulf War, but these findings were not confirmed. Interference from petroleum products and oil fires explains the false readings (OSAGWI, 1997c).

Phosgene Oxime (Agent CX)

Phosgene oxime, which was developed before World War II, has not been studied extensively. Iraq may have used it against Iran (OSAGWI, undated a). This chemical is highly reactive and unstable, making it an unlikely hazard if transported long distances through the atmosphere. It is unusual in that it can harm materials as well as people. Phosgene oxime is very painful and irritating, especially to the skin and eyes, so exposure is likely to be noticed. Higher levels of exposure cause severe skin burns that heal slowly and may result in fatal pulmonary edema. No information on the effects of long-term or low-level exposure to phosgene oxime was found in the unclassified literature. In an incident after the Gulf War, a coalition officer sustained a severe, painful chemical burn that was suspected of being due to exposure to phosgene oxime, but was later determined to be from some kind of nitric acid (OSAGWI, 1998a).

Mustards (Agents H, HD, T, HT, Q, and HN)

The mustard agents (first used during World War I) have similar chemical structures and are effective military agents. Their mechanism of action is not fully understood, but it is known that they interact with DNA to cause cell death in ways that resemble radiation injury. This review concentrates on sulfur mustard (H), which Iraq used widely against Iran (UN, 1984). Mustard agents are quite persistent and stable in the environment. Because of the delayed onset of effects (as much as a day), exposure is not immediately obvious. Iraq was thought to have used a "dusty" form of mustard (mustard absorbed on fine silica particles) in its war with Iran. This form is said to produce more-rapid skin

effects and more-lethal pulmonary injury and may be more difficult to detect (OSAGWI, 1996).

Mustards are primarily incapacitating agents, producing skin blisters and eye injuries that are disabling for a time, although recovery usually occurs. More-severe pulmonary, skin, and systemic poisoning can be fatal. Mustards injured thousands of soldiers during World War I. While some of the injured had respiratory complaints, the other complaints associated with Gulf War illnesses were not noted. With the repeated exposures munitions workers encounter, serious delayed effects can occur. Long-term mustard workers are in poorer health than their peers, reporting depression, nervousness, autonomic disorders, bronchitis, and frequent infections. They have a higher prevalence of skin and lung cancer (Yamakido et al., 1996; Pechura and Rall, 1993; Dacre and Goldman, 1996; Lohs, 1975).[5]

Low-level exposure to mustards can produce skin erythema resembling sunburn, with eye irritation, runny nose, sore throat, cough, and lethargy, which could be misdiagnosed or overlooked. However, loss of taste and smell is commonly associated with respiratory symptoms, which is not typical of colds or irritants. Scrotal inflammation strongly suggests mustard exposure.

There are a number of Iranian reports on the results of civilian exposures to mustards. These include changes in male-to-female birth ratios (Pour-Jafari, 1994) and increases in cleft lip and other congenital malformations (Taher, 1992). However, other environmental and nutritional factors could not be excluded. Mustards suppress the immune system but are immunogenic in their own right, including hypersensitivity reactions to later exposures (Grunnet, 1976).

Although early clinicians thought that mustards injured the nervous system, the neurological effects of mustard exposures have not been well studied (Vedder, 1925, Dacre and Goldman, 1996). It is known that mustards inhibit cholinesterase, and early mustard casualties were depressed and lethargic. Recently, there have been reports of a high prevalence of post-traumatic stress disorder (PTSD) in veterans who were subjects of World War II experimental mustard exposures (Schnurr, Friedman, and Green, 1996). The matter is still under study, and the mechanism of the disorder is not understood.

There is evidence that combined exposures to mustards and nerve agents have greater toxicity than expected from either alone (Krustanov, 1962). There are no data on combined effects of mustards and pyridostigmine bromide.

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[5]Mustards are mutagens and carcinogens, but the risk from brief low-level exposures is small (Pechura and Rall, 1993; Dacre, 1996).

There are now sensitive measurement techniques to detect mustard adducts with hemoglobin and with DNA in tissues (Ludlum and Austin-Ritchie, 1994; Ehrenberg and Osterman-Golkar, 1980). It may be possible to detect such effects in the blood and tissues obtained during and after the Gulf War and now housed at the Armed Forces Institutes of Pathology (AFIP).

BIOLOGICAL TOXINS

Biological toxins are poisons produced by living organisms. The most potent poison known is botulinum toxin, a toxin reviewed in the RAND infectious disease report (Hilborne et al., 2000). The toxins reviewed in this report are ricin (which is extracted from the castor bean) and the trichothecene toxins and aflatoxins (which are produced by fungi). All of these toxins are capable of producing fatal illnesses that are virtually untreatable. None of these agents could be detected by the systems available to U.S. forces during the Gulf War.[6] Therefore, clinical recognition was the means of toxin detection. We are unaware of reports of illness attributed to toxins.

More is known about ricin and the trichothecene toxins as military agents than about aflatoxin, which was considered a public health problem until it was revealed after the war that Iraq had placed aflatoxin in missiles and rockets (Zilinskas, 1997).

Ricin (Agent W)

Ricin is a protein that can be extracted from the castor bean. Its toxicity has been known and used since ancient times. Although it was not used in World War II, it was developed to weapon status by the United Kingdom and the United States during that war (OSRD, 1946). Ricin is a powerful weapon—the same dose of a crude ricin aerosol extract or the nerve agent sarin both kill 50 percent of the exposed population.

Ricin is not stable in the environment (degrading in a few days), so it is unlikely that ricin would be a threat after long-distance travel through the atmosphere. Effective military attack with ricin would produce, after a delay of hours, eye inflammation, severe pulmonary edema, and death from respiratory failure, as is seen in nonhuman primates exposed to ricin aerosols (Wilhelmsen, 1996). When injected, ricin produces a fever, high white blood cell count, seizures, and multiple organ failures. It does not apparently damage or penetrate the skin.

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[6]Specialized units were deployed to collect, sample, and evaluate biological agents possibly used during the war, but in some cases, material was referred to U.S. laboratories for analysis.

The clinical effects of low-level inhalation exposure were observed in research and production workers in World War II (OSRD, 1946). Symptoms of coughing, dyspnea, inflammation and burning of the trachea, aching joints, and nausea began four to eight hours after exposure. Several hours later, profuse sweating usually preceded the abatement of symptoms. Muscle cramps and weakness are common after ricin exposure. No long-term follow-up information was found.

Ricin is highly immunogenic. Laboratory workers responded to second exposures with apparent hypersensitivity reactions—sneezing, coughing, and other asthmatic symptoms. If U.S. forces were exposed to ricin, it should be possible to find ricin antibodies in the exposed persons. Iraq admitted testing it in artillery shells but claimed not to have deployed it as a weapon (Zilinskas, 1997).

Trichothecene Mycotoxins

This family of toxins was used as military agents in Southeast Asia, where they were referred to as "yellow rain," and later in Afghanistan, where they were used by Soviet forces. Iraq admitted to the UN that it had produced some trichothecene toxins and tested them on animals but contended that they had not proceeded to weapon development (Zilinskas, 1997). There were reports that Iraq had used these toxins against Iran (such as Heyndrickx, 1984, pp. 132–146), but other laboratories could not detect trichothecene mycotoxins in Iranian casualties. The toxins are very stable in the environment and resist decontamination, so continued toxicity after long-distance atmospheric transport is possible. There are many tricothecene mycotoxins, and it is not known which ones Iraq produced.

Trichothecene toxins (T-2 being the most important and most studied) can be lethal when inhaled or ingested and are damaging to the skin and eyes at very low concentrations (nanograms to micrograms). Vomiting and nervous system effects after low-level exposures are common.

After substantial acute exposures (cancer therapy; yellow rain), sequelae have included rashes, joint pain, fatigue (Schultz, 1982), fever, chills, hypotension, confusion, somnolence, memory loss, hallucinations (Belt et al., 1979), dyspnea, diarrhea, and recurrent infections (Yap et al., 1979; Murphy et al., 1978; Crossland and Townsend, 1984). Laboratory findings show low white-cell counts, low platelet levels, and decreased coagulation factors.

Chronic low-level exposure of a family in a house contaminated with tricothecene mycotoxins (probably verrucarin, roridan, and satratoxin) produced frequent respiratory illness, flu-like symptoms, sore throat, diarrhea, headache, fatigue, and alopecia (hair loss). The family had marked changes in hematolog-

ical values. These problems defied diagnosis for a long time (Croft, Jarvis, and Yatawara, 1986; Jarvis, 1985).

Memory loss has been seen in survivors of yellow rain, in cancer chemotherapy patients, and in tests on experimental animals. Such cases were usually accompanied by hematological changes, which have not been an apparent feature in Gulf War veterans suffering from Gulf War illnesses.

If trichothecenes were prevalent in the Gulf theater environment, one would expect a high prevalence of eye irritations and skin inflammations, since these agents act on the skin and eyes so powerfully at low concentrations (nanogram to microgram amounts).

It is theoretically possible to detect trichothecene metabolites in tissues collected during and after the Gulf War. These tissues are available to the AFIP. Since trichothecene exposure can occur naturally, suitable controls and a sophisticated experimental design would be required. Such studies would be technically difficult. The views of the AFIP on this matter should be determined.

Aflatoxins

Because aflatoxins can contaminate food and grain, causing illness in domestic animals and perhaps liver cancer in humans, they have long been of concern as a public health threat. Before the Gulf War, aflatoxins (of which the type designated AFB_1 is the most toxic) had not been used in war and had received only passing mention in discussions of biological warfare (U.S. Army, 1990). Thus, the report that Iraq had produced substantial amounts of these toxins to fill Scud missiles and 122 mm rockets was surprising. The intended effect is unknown, and some analysts were uncertain about why these toxins were selected as a weapon (Zilinskas, 1997).

These toxins are very stable, are resistant to decontamination, and would retain their toxicity after long-distance atmospheric transport (although they would be diluted in concentration). They do not represent a hazard to the skin, which appears to be an effective barrier against them. Since metabolic activation is required for their toxicity to manifest itself, their effects are delayed for several hours after exposure.

Experience with heavily contaminated food indicates that human acute lethal doses are in the range of 2 mg/kg (Harrison and Garner, 1991). The toxin is well absorbed from the lungs, and it would be feasible to create an aerosol to deliver 140 mg, a fatal dose for a 150-pound man. Orally intoxicated humans acutely show vomiting, seizures, fever, respiratory distress, liver failure, and coma (Chao et al., 1991; Bourgeois, Olson, et al., 1971). There are no follow-up data from the known acute oral poisonings.

We do not have a clinical picture of low-level respiratory exposure to aflatoxins. One could expect nausea, vomiting, cough, and respiratory symptoms. Animal studies indicate that inhaled aflatoxins at low levels are immunosuppressive (Jakab et al., 1994). However, grain workers exposed to such toxins are not reported to have high prevalence of pulmonary infections (Autrup, Schmidt, and Autrup, 1993). Indeed, grain workers who encounter these toxins also have other complex exposures and primarily report nonspecific respiratory symptoms.

Some animal data hint at respiratory toxicity from low doses. Although no primate aerosol studies were found, other animal studies have shown pulmonary damage from nanogram and microgram amounts (Northup et al., 1995; Jakab et al., 1994).

Chronic oral intake of low concentrations of aflatoxins is associated with increased risk of liver cancer, impaired child health and development, and frequent infections (Groopman, Scholl, and Wang, 1996). Neurological problems have not been described. The increased cancer risk from short-term exposure is small.

Persons exposed to aflatoxins develop antibodies to the toxin. It is possible to identify aflatoxins and their adducts in formalin-fixed tissue of poisoned victims (Harrison and Garner, 1991). Whether it would be possible to detect the toxin in tissues available to the AFIP is a matter for discussion with that organization. The expense and complexity of such studies do not commend them as a screening method, but such studies might be valuable if there are indications of possible exposure. Care in the design of such studies is essential, since western European populations have been shown to have low levels of these antibodies (Autrup and Seremet, 1990).

NERVE AGENTS

The nerve agents are part of a group of organophosphorus compounds, which are potent inhibitors of the enzyme acetylcholinesterase. This enzyme regulates neural function by inactivating the neurotransmitter acetylcholine. This group of chemicals also includes organophosphate pesticides, which are chemically similar to the nerve agents but less toxic.[7]

This study reviewed the nerve agents tabun (GA), sarin (GB), soman (GD), cyclosarin (GF), and VX.[8] The agents reviewed differ in various respects. Tabun is

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[7]Another class of chemicals can also inhibit acetylcholinesterase: the carbamates, of which pyridostigmine bromide is a member.

[8]We attempted to review thiosarin, an analog of sarin that replaces the oxygen attached to the phosphorus molecule with sulfur, but could find little information on this chemical.

the least toxic. Sarin is highly volatile, which makes it less of a skin-exposure hazard. Soman is of intermediate volatility and is resistant to treatment. VX is potent but not volatile. Cyclosarin is more persistent than sarin. Still, there are sufficient similarities in the biochemistry of these agents to treat them as a group in this review. Although the class of agent would be evident, it is unlikely that a clinician seeing a mild or severe casualty from a nerve agent would be able to discern which agent produced the illness.

The Germans discovered nerve agents shortly before World War II. This class of chemicals remains the most toxic ever known. Two agents (tabun and sarin) reached weapon status, but were not used, during World War II. In the years after World War II, hundreds of military agents and thousands of pesticides in this class were tested. There is considerable information about the effects of these agents on humans from accidents during research, development, and production; from warfare; and from studies on volunteers. Sarin is the best documented and cyclosarin the least well documented. There is an enormous literature about the effects of nerve agents on animals and *in vitro*.

Militarily efficient attacks with vapor or aerosols have very rapid effects, including collapse, respiratory distress and failure, seizures, and paralysis. Less severely exposed persons experience confusion, dim vision, respiratory difficulty, marked salivation, vomiting, diarrhea, weakness, tremors, and incoordination. Milder exposures produce acute symptoms including dim vision (impaired night vision is a distinctive result), difficulty and pain with focusing, headache, red eyes, runny nose, tight chest, and cough, followed by nausea, cramps, diarrhea, and muscle weakness. Confusion, irritability, depression, difficulty thinking, poor coordination, and sleep disturbances are also common. Although not extensively studied, people who have been exposed to nerve agents are known to be accident prone, although most seem to recover from this in a few days or about two to three weeks (Gaon and Werne, 1955; Holmes, 1959).

During research and production of these nerve agents, it was noted that some persons who did not report illness were found to have very low levels of acetylcholinesterase, indicating unrecognized exposure. Such persons have not been the subjects of long-term follow-up studies. A study of agricultural workers exposed to organophosphorus pesticides detected impaired mental performance in workers who reported no symptoms and whose exposure to these pesticides was documented (Stephens, Spurgeon, and Berry, 1996).

Iraq used tabun against Iranian forces in the Iran-Iraq war (UN, 1984) and may have used other nerve agents (sarin, cyclosarin) later in that war (OSAGWI, undated e). It was known that Iraq conducted research on and had chemical agents, but there remains no absolute certainty about the numbers or types of Iraqi chemical and biological weapons that were in existence at the time of the

Gulf War. (It was several years after the war that the UN documented Iraqi possession of VX, tabun, sarin, and cyclosarin). Before the war, it was suspected that Iraq had stocks of soman, but this has not been proven. Exposure to this agent is hard to treat, and this was the threat that inspired the use of pyridostigmine bromide. There may have been some low-level exposures of U.S. forces to sarin and cyclosarin after the war as a result of the demolitions at Khamisiyah. The CIA estimated that any exposure was an amount unlikely to produce any acute clinical effect (CIA, 1997; OSAGWI, 1997a).

Longer-Term Effects

Both human and animal research data indicate that low-level exposures to nerve agents have long-term effects. The prevailing view of experts in the 1950s and 1960s was that complete recovery always occurred following nerve agent exposure (other than in severe cases in which anoxic brain damage occurs) (Grob, 1956). This view is supported by the fact that no long-term health problems due to agent exposure were seen in volunteers exposed to the agents (NRC, 1985). The prevalent view may have decreased the recognition of longer-term effects, although these were occasionally reported (Gaon and Werne, 1955; Craig and Freeman, 1953). Furthermore, the Japanese experience with moderate and mild exposures indicates that subtle neurological dysfunction can be detected at six months and a year for some individuals (Yokoyama et al., 1998; Nakajima, Ohta, et al., 1998).

Nonhuman primates showed electroencephalogram (EEG) changes lasting over a year following a short series of doses of sarin, which did not make them sick (Burchfiel and Duffy, 1982). Longer-term studies of production workers exposed to sarin indicated that muscle aches and pains, drowsiness, and fatigue were significant problems. Continued study of others a year after their last exposure showed disturbed memory, difficulty in maintaining alertness and attention, and "soft" neurological findings suggesting coordination defects. Computer-interpreted EEGs were abnormal (Metcalf and Holmes, 1969; Duffy and Burchfiel, 1980).

Delayed Neuropathy

Some organophosphorus compounds produce a delayed injury to the nervous system that becomes obvious weeks to months after exposure. This produces paralysis from a peripheral neuropathy, although occasionally effects may resemble other neurological disorders (Hayes, 1982; Johnson, 1975; Abou-Donia, 1981). There is no treatment. Inhibition of an enzyme, neuropathy target es-

terase (NTE), is required to produce the neuropathy.[9] There had been concern that nerve agents might produce this disorder. However, it was only possible to produce delayed neuropathy in animals when they were dosed with levels of sarin and soman many times the LD_{50} dose (it was necessary to treat the animals to keep them alive) (Gordon, Inns, et al., 1983). More recently, Husain, Vijayaraghavan, et al. (1993) have shown that mice exposed to ten daily doses of sarin that did not make them sick from anticholinesterase effects had typical delayed neuropathy lesions.

Although the main weight of the evidence suggests that nerve agents have little ability to produce delayed neuropathy, the complexity of this phenomenon and the complexity of the interactions with other chemicals make it impossible to reject it on clinical grounds alone and suggest more research is needed in this area.[10]

Tolerance and Other Adjustments to Exposure

Exposures to a variety of chemicals that inactivate acetylcholinesterase (even when not clinically apparent) can induce a condition of tolerance, in which signs and symptoms of further acute exposure are greatly diminished or lacking. This condition is associated with a decrease in the abundance and sensitivity of acetylcholine receptors (Costa, Schwab, and Murphy, 1982). This condition may also be a pathological process with impairments in memory and learning (Taylor, El-Fakahony, and Richelson, 1979; Buccafusco et al., 1997). Although humans have shown tolerance to pesticides and nerve agents, we have no information about the recovery from this condition. Repeated exposures to anticholinesterases (such as pesticides or pyridostigmine bromide) could induce a condition of tolerance that would make clinical recognition of low-level nerve agent exposure more difficult and that would further increase the level of tolerance. Tolerant animals and people are very sensitive to the effects of anticholinergic drugs such as atropine, antihistamines and other like drugs (Chippendale et al., 1972).

Anticholinesterase chemicals may produce longer-term changes in brain chemistry by affecting the expression of immediate early genes (such as c-fos) and the proteins they encode. Alternation of the expression of immediate early genes can affect how many other genes respond to environmental stimuli. Kaufer et al. (1998) observed that, in animals, robust cholinergic stimulators

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[9]The function of NTE is unknown; it is found in the spinal cord, brain, sciatic nerve, platelets, and lymphocytes.

[10]As of late 1999, no findings of typical axonal neuropathy have been reported in Gulf veteran studies.

(including stress, pyridostigmine bromide, pesticides, and nerve agents) can all induce increased expression of c-fos in the brain. Kaufer et al. contend that alternation of c-fos expression may induce "convergent" mechanisms that contribute to longer-term brain effects and specifically mention Gulf War illnesses. Developments in this field deserve close attention, although the duration and consequence of such changes in gene expression and their clinical significance have not yet been determined.

Possible Long-Term Consequences from Unrecognized Exposure

Earlier reviews generally discounted a role for nerve agent exposures in contributing to undiagnosed illness in Gulf War veterans because no recognized exposures had produced signs and symptoms. This review identified biological mechanisms that can sequester or actively degrade nerve agents, so there is some (unknown) level of agent exposure that would have no effect because of detoxification. The levels of exposure calculated from the Khamisiyah event are so low that enduring responses would not be expected, and indeed, the exposure may have been at the no-effect level (CIA, 1997).

Still, low-level exposures to nerve agents are difficult to recognize. Animal research and human experience with nerve agents and organophosphorus pesticides show altered neurological function from doses that do not produce overt signs or symptoms (Gaon and Werne, 1955; Brody and Gammill, 1954; Craig and Freeman, 1953; Bowers et al., 1964; Korsak and Sato, 1977; Sirkka, Nieminen, and Ylitalo, 1990; Wolthuis, Groen, et al., 1990; Stephens, Spurgeon, and Berry, 1996; Burchfiel, 1976). Indeed, mild to moderate nerve agent exposures seem capable of producing biological changes and health effects lasting over a year. No reports were found in the literature of disorders attributed to nerve agents that began two or more years after exposure.

Some long-term follow-up studies of nerve agent exposures report signs and symptoms similar to those reported by those suffering from Gulf War illnesses (Metcalf and Holmes, 1969). Japanese reports a year after exposure do not indicate similar problems, although there are subtle neurological findings and persisting eye pain and irritation (asthenopia) (Yokoyama et al., 1998a, b; Nakajima, Ohta, et al., 1998).

There is little evidence to asses the long-term consequences of exposure to levels of nerve agent that are too low to produce any acute effects. This possibility remains an area needing further research

It may be possible to determine directly if soldiers were exposed to nerve agents during the Gulf War. Measurement of nerve agent metabolites in formalin-fixed tissues has been reported in Japan (Matsuda et al., 1998). Whether these

techniques are sensitive enough to detect metabolites from low-level exposures in tissues available to AFIP is uncertain.

STRESS

Responses to stress are the subject of another RAND review (Marshall, Davis, and Sherbourne, 1999). However, because the threat of chemical and biological agents arouses concern in most people, this review of such agents also touches on this topic.

The operational activity to prepare for chemical and biological attacks during the Gulf War occupied considerable training and other activities involving most personnel (Clancy and Franks, 1997). Many persons find wearing the necessary protective equipment to be physically demanding (Teitlebaum and Goldman, 1972; Joy and Goldman, 1964) and psychologically stressful (Brooks et al., 1983, Carter and Cammermeyer, 1985). During the war, agent alarms and other precautions raised the possibility of chemical and biological weapons attack and caused personnel to wear full protection for sustained periods. These events were certainly the source of stress for some individuals.

It is not easy to separate late clinical effects of agent exposure from late stress effects of PTSD. There are indications that veterans who participated in World War II mustard experiments have a high prevalence of PTSD (Horvath, 1997; Schnurr, Friedman, and Green, 1996). The Hmong survivors of trichothecene attacks (yellow rain) in Southeast Asia showed prolonged problems of apathy, anxiety, and disordered memory (they had also been severely traumatized by attacks and harrowing refugee experiences) (Crossland and Townsend, 1984). PTSD has been seen in survivors of the Japanese sarin attacks and has complicated analyses of other subtle late effects of nerve agent exposure (Yokoyama, Araki, et al., 1998a, 1998b).

Animal data indicate that the use of steroids can potentially alter responses to nerve agents and vice versa (Clement, 1985; Stabile, 1967). There is evidence, which has been challenged by some recent studies, that stress in animals can increase the permeability of the blood-brain barrier (Sharma, 1991), permitting entrance to the brain of chemicals normally excluded. Although the full clinical significance has yet to be determined, the observation in animals that stress and several anticholinesterases (including pyridostigmine bromide, nerve agents and pesticides) have a common reaction mechanism (promoting the expression of the immediate early gene c-fos in the brain) is noteworthy (Kaufer et al., 1998).

CONCLUSIONS AND FURTHER RESEARCH

This review describes the properties and health effects of chemical and biological agents that might have been available to Iraq at the time of the Gulf War. The review is intended to provide information on the health effects of these chemical warfare agents and toxins, and to help determine on clinical grounds if personnel were exposed to these agents during and shortly after the Gulf War. The review found gaps in the literature, including inadequate or no useful information on thiosarin, long-term follow-up on exposure to phosgene oxime and the toxins, and follow-up data on persons who had become tolerant of nerve agents. Additionally recommended research areas are provided at the end of this section.

A number of conclusions can be drawn from the study:

1. Militarily effective exposure to any of the agents reviewed would have produced severe health effects that would have required clinical treatment or resulted in death. Although some differential diagnosis between agents might have been difficult, no such symptoms consistent with large-dose exposure were reported during the Gulf War, with the exception of the possible exposure of one individual to mustard gas residual in an Iraqi bunker.
2. Low-level exposure to many of these chemical weapon agents could have produced mild clinical signs that could have been overlooked or misinterpreted as arising from other common sources, such as irritation from dust and sand, upper respiratory infections, gastroenteritis, asthma, and the flu. The possibility of low-level exposure to a number of agents more or less at the same time makes diagnosis very difficult. There can be problems of recognition, of modifications of response, or of distinct effects from unrecognized exposures. Therefore, on clinical grounds alone, it is not possible to rule out low-dose exposures to one or several classes of agents or the possibility of some resultant contribution to some of the symptoms Gulf War veterans have experienced. Still, it is difficult to believe that exposures affecting large numbers of persons would escape clinical recognition.
3. No references in the literature report clinical symptoms developing years after exposure, as was the case in about 50 percent of the health problems Gulf War veterans have reported.
4. There is very little literature on the long-term effects of exposure to doses below those that would cause any acute clinical symptoms. The possibility that such exposure could possibly produce chronic health effects cannot be ruled out based on the current state of the literature and needs to be investigated, both for one-time exposure and for longer-term, very-low-dose exposures over an extended period.

The report also reviewed some literature on the possible interaction effects of chemical warfare agents and other factors. Kaufer et al. (1998) discuss several factors that may contribute to symptoms seen in Gulf War veterans—including stress, pyridostigmine bromide, and anticholinesterase nerve agents—are capable of activating regulatory genes (such as c-fos) in the brains of animals. Because of the potential long-term effects of these genes, this observation may be important. However, the long-term effects in animals and the clinical significance of these studies for humans remain to be determined. Epidemiological studies of illnesses in veterans of the Gulf War might consider the possibility of aggregate effects arising from the varied factors noted above. Since mustards and trichothecene toxins (as well as nerve agents) also inhibit acetylcholinesterase, it may be useful to examine their effects on regulatory gene expression as well.

Finally, there are significant gaps in our knowledge that warrant additional research. The most obvious area in need of a great deal of additional research is the health effects from exposure to low levels of chemical warfare agents and toxins. Little is known about the long-term effects of either a single exposure or longer-term exposures to low doses of chemical warfare agents and toxins that result in either mild symptoms or no acute symptoms at the time of exposure.[11]

Further research in many other areas would also be very useful; these areas include the following:

- A better understanding is needed of the effects of mustard agents on the functioning of the central nervous system, including the brain effects of low doses of mustard agents in combination with nerve agents and pretreatments.
- Long-term follow-up studies of the Japanese exposed to sarin—particularly at low levels—in the subway attack would be useful.
- A better understanding is needed of the acute respiratory toxicity of aflatoxin in nonhuman primates, as well as a better understanding of aflatoxin's role in seizures and neurotoxicity. Follow-up information from survivors of the Malaysian poisoning event would be valuable.
- Research is needed to determine the long-term effects of converging cholinergic activation of c-fos. Likewise, the understanding of the consequences of receptor downregulations from cholinergic stimuli must be improved.

[11]It should be noted that the Department of Defense and the Veterans' Administration also recognize this need and have initiated a good deal of such research.

- The work of Kaufer et al. (1998) on the duration of the response in the convergent response mechanism of nerve agent action should be followed up, and the relative effects of low levels of known nerve agents in this model should be documented.
- The observations of Buccafusco et al. (1997) on the downregulation of nicotinic receptors in the brain from low-dose diisopropyl fluorophosphate (DFP), with subsequent impaired learning in animals, needs to be extended. Confirming the effect with low-dose nerve agents and documenting the duration of the effect appears to be important. Primate studies with more-complex performance studies could follow.
- Those involved in epidemiology studies of accidents during and immediately after the Gulf War should be made aware of the observation that sarin workers who recovered from mild exposures were noted to have many industrial and vehicular accidents.
- The capabilities of the AFIP may help rule in or out certain exposures. Autopsy and surgical material from the Gulf War and from veterans later might be reviewed, looking for tissue evidence of various agent exposures (which, since they were not an obvious cause of death, might have been overlooked in initial studies). There are indications that biochemical evidence of exposure to various agents can be detected in blood serum and even in formalin-fixed tissues. It is far from certain that existing methods are sensitive enough to identify low-level effects (e.g., DNA adducts in formalin-fixed tissues from autopsies). Whether to conduct such studies is not simple, since the expense does not commend such studies as a screening method. Perhaps such studies should be reserved for cases in which suspicion is high. There may be reasons beyond Gulf War illnesses to develop such a capability.