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GULF WAR and HEALTH

Updated Literature Review of Sarin

Committee on Gulf War and Health: Updated Literature Review of Sarin

Board on Health Promotion and Disease Prevention

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OF THE NATIONAL ACADEMIES

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Willing is not enough; we must do."
—Goethe

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COMMITTEE ON GULF WAR AND HEALTH: UPDATED LITERATURE REVIEW OF SARIN

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This report has been reviewed in draft form by persons chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the National Research Council's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards of objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following for their review of this report:

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Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations, nor did they see the final draft of the report before its release. The review of

this report was overseen by **Lauren A. Zeise,** Chief, Office of Environmental Health Hazard Assessment, Reproductive and Cancer Hazard Assessment Section, California Environmental Protection Agency. Appointed by the National Research Council and Institute of Medicine, she was responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

Preface

Following the Iraqi invasion of Kuwait in August of 1990, approximately 700,000 US troops were deployed to the Persian Gulf in 1990 and 1991. Although the duration of combat was short and casualties few, upon their return many of the Gulf War veterans began experiencing unexplained symptoms, such as muscle and joint pain, fatigue, difficulties of cognition, and headaches. Studies have shown that the prevalence of these symptoms clearly was higher among veterans who had been deployed to the Persian Gulf than among either those not deployed or those sent to other wars. This has led many to consider the possibility that exposures unique to the Persian Gulf Theater could be the source of the illnesses.

In 1998, in response to the health concerns of veterans and their families, the Department of Veterans Affairs contracted with the Institute of Medicine (IOM) to study the scientific evidence concerning possible adverse health effects of multiple agents to which veterans may have been exposed. To carry out this assignment, the IOM has convened three committees. The first committee report addressed the effects on health of four sets of compounds: depleted uranium, sarin and cyclosarin, pyridostigmine bromide, and vaccines against botulinum toxin and anthrax. The second committee reported on the health effects of exposure to insecticides and solvents. The third committee is currently reviewing the combustion products of oil-well fires, fuels, and compounds potentially used as propellants for Scud missiles.

Our ad hoc committee was asked to update the first committee's report on outcomes of exposure to sarin and cyclosarin, in light of more recent studies of sarin exposure from terrorist attacks in Japan; possible sarin exposure of veterans

at Khamisiyah, Iraq, during the Gulf War; and more recent toxicological studies on low-dose exposure to sarin. In as much as no veterans of the Gulf War are known to have had symptoms of acute sarin toxicity, our focus was on the long-term effects of low-dose exposure. In addition, since sarin and cyclosarin are strong acetylcholinesterase inhibitors, we also reviewed recent studies on organophosphorus insecticides, which also are cholinesterase inhibitors.

I am deeply appreciative of the fine work and great expertise of committee members, William Daniell, MD, MPH; Rose Goldman, MD, MPH; Richard Mayeux, MD, MSc; Samuel Potolicchio, MD; and Joseph Rodricks, PhD. Further, the study could not have been successfully completed without the superb efforts of study director Michelle Catlin and research assistant Deepali Patel.

Jack M. Colwill, MD

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Executive Summary

The Gulf War in 1990–1991 was considered a brief and successful military operation, with few injuries or deaths of US troops. The war began in August 1990, and the last US ground troops returned home by June 1991. Although most Gulf War veterans resumed their normal activities, many soon began reporting a variety of nonexplained health problems that they attributed to their participation in the Gulf War, including chronic fatigue, muscle and joint pain, loss of concentration, forgetfulness, headache, and rash.

Because of concerns about the veterans' health problems, the Department of Veterans Affairs (VA) requested that the Institute of Medicine (IOM) review the scientific and medical literature on the long-term adverse health effects of agents to which the Gulf War veterans may have been exposed. Congress also passed legislation for a similar study (the Persian Gulf War Veterans Act of 1998, PL 105-277, and the Veterans Programs Enhancement Act of 1998, PL 105-368).

In response to those requests, IOM has convened committees to evaluate the health effects of various chemicals used in the Gulf War. The first evaluated the health effects of depleted uranium, pyridostigmine bromide, sarin and cyclosarin, and vaccines (anthrax and botulinum toxoid) and produced *Gulf War and Health, Volume I*, hereafter referred to as *GW1*. The second reviewed the health effects of solvents and pesticides and produced *Gulf War and Health, Volume 2*, hereafter referred to as *GW2*. Another committee is reviewing the health effects of the combustion products of oil-well fires, fuels, and synthetic compounds potentially used as propellants for SCUD missiles.

Because of continued concerns of veterans, especially in light of recent toxicologic studies of low-dose exposure to the chemical-warfare agent sarin, the

VA requested that IOM conduct an updated evaluation of the health effects of sarin. A new committee, made up of some of the members of the committee responsible for *GW2*, was convened to conduct this review.

CHARGE TO THE PRESENT COMMITTEE

The present committee was charged to review the peer-reviewed literature published since earlier IOM reports on health effects associated with exposure to sarin and related compounds, including relevant epidemiologic studies. With regard to the toxicologic literature, the committee used review articles to obtain and present a broad overview of the toxicology of sarin and cyclosarin, and to assess biologic plausibility with respect to the compounds in question and health effects; individual toxicologic research papers were evaluated as warranted. The committee based determinations on the strength of the evidence of associations between the compounds and human health effects. If published, peer-reviewed information was available on the magnitude of sarin and cyclosarin exposure of Gulf War veterans, the committee addressed the potential health risks posed to the veterans. The committee also considered other relevant issues, such as exposure to multiple chemicals and genetic susceptibilities. The committee's review included recommendations for additional scientific studies to resolve continued scientific uncertainty as warranted.

The committee was not charged with determining whether a unique Gulf War syndrome exists, nor was it to make judgments regarding magnitudes of exposure of veterans to the putative agents. Moreover, the committee was not charged to focus on broader issues, such as the potential costs of compensation for veterans or policies regarding such compensation. Those decisions remain the responsibility of the secretary of veterans affairs. This report does, however, provide an assessment of the scientific evidence regarding health effects that may be associated with exposures to specific agents that were present in the Gulf War. The secretary may consider those health effects as the VA develops a compensation program for Gulf War veterans.

APPROACH TO THE CHARGE

The committee's first step was to identify the literature to be reviewed. The search was conducted by using the names of sarin and cyclosarin and their synonyms. Titles and abstracts were reviewed to determine their relevance to the committee's charge; potentially relevant studies were retrieved and evaluated. The literature was also searched for epidemiologic studies on organophosphorus (OP) compounds published and catalogued since August, 1999, when the last search was conducted for the preparation of *GW2*, and such studies were reviewed.

Animal studies had a small role in the committee's assessment of association between putative agents and health outcomes. In general, animal data were used

for making assessments of biologic plausibility in support of the epidemiologic data rather than as part of the weight of evidence to determine the likelihood that an exposure to a specific agent might cause a long-term outcome.

The committee classified the evidence of an association between exposure to sarin and cyclosarin and a specific health outcome into five categories: sufficient evidence of a causal relationship, sufficient evidence of an association, limited/suggestive evidence of an association, inadequate/insufficient evidence of an association, and limited/suggestive evidence of no association. The categories are modified from established categories of association from previous IOM studies that have gained wide acceptance over more than a decade by Congress, government agencies, researchers, and veterans groups.

POTENTIAL US TROOP EXPOSURE TO SARIN AND CYCLOSARIN

Sarin (GB; *o*-isopropyl methylphosphonofluoridate) and cyclosarin (GF; cyclohexyl methylphosphonofluoridate) are highly toxic OP nerve agents produced for chemical warfare. During a cease-fire period in March 1991, a large storage complex at Khamisiyah, Iraq, was destroyed. Two sites in the complex contained rockets loaded with sarin and cyclosarin. The total amount released, according to the most recent estimates, is 371 kg of sarin and cyclosarin combined. US troops performing demolitions were unaware of the presence of nerve agents, and no air monitoring was conducted at the time of the demolition.

Modeling has been conducted to determine potential exposures of US troops. According to model estimates, no troops were exposed to doses greater than would cause "first noticeable effects", which would set off chemical alarms and cause visible signs of the acute cholinergic syndrome. There were no medical reports by the US Army Medical Corps at the time of the release that were consistent with signs and symptoms of acute exposure to sarin. That information is consistent with the absence of reports of symptoms of an acute cholinergic syndrome by medical personnel or veterans.

TOXICITY, EXPERIMENTAL ANIMAL AND MECHANISTIC DATA

Neurotoxicity

OP compounds are absorbed rapidly and produce local and systemic effects. Clinical signs of toxicity associated with organophosphate-induced inhibition of acetylcholinesterase (AChE) depend on dosage. Toxicity in humans and animals includes the signs associated with overstimulation of muscarinic receptors[1] of the

[1]Muscarinic receptors are a subtype of receptors to which acetylcholine (ACh) binds. Binding of ACh to muscarinic receptors activates those receptors. Excessive activation of those receptors can lead to overstimulation of muscles and nerves.

autonomic nervous system by acetylcholine (ACh)—salivation, sweating, meiosis, tremor, lacrimation, urination, defecation, emesis, and bradycardia.

The principal mechanism of acute toxicity of sarin and cyclosarin, like that of other OP compounds, is inhibition of AChE. AChE is responsible for the hydrolysis of ACh at the synapse, and inhibition of that enzyme leads to a rise in ACh and overstimulation at cholinergic synapses. Obvious signs of the acute cholinergic syndrome do not generally appear until nervous system AChE inhibition approaches 70%. Some effects of sarin, however, do not appear to be related to inhibition of AChE.

Long-term changes in the electroencephalogram (EEG) of rhesus monkeys have been seen after a single high dose of sarin or a series of 10 small doses. The high dose was sufficient to produce the acute cholinergic syndrome, whereas each small dose produced few, if any, signs of acute poisoning. Changes persisted for 1 year after sarin administration, although the changes did not appear to have any behavioral or psychologic significance. Similar effects were not seen in marmosets or in guinea pigs except when they were exposed to doses that caused the acute cholinergic syndrome.

Recently, research has been conducted to resemble more closely the sarin exposures that might have occurred in the Gulf War. The highest concentration tested was one-tenth the lethal concentration. No consistent effects on locomotor activity or body temperature of rats were seen. Brain was examined histopathologically 30 days after exposure; no lesions or evidence of cell death were present. No effect was seen on total brain AChE measurements, but AChE was decreased in some brain regions—mostly in areas of the forebrain (the cerebral cortex, striatum, olfactory bulb, and the CA1 region of the hippocampus). Brain cytokine concentrations were affected by both sarin treatment and heat stress. Receptor density was measured for the M1, M2, and M3 subtypes of muscarinic receptors. M1 receptors were decreased in a dose-dependent manner in some brain regions. Sarin did not affect M3 receptor density under normal conditions, but under heat stress there was an increase in the number of M3 receptors in some brain regions. Some of those changes remained for the duration of the experiment (30 days). The results related to receptor density are suggestive of a potential mechanism through which sarin could cause long-term effects on the nervous system and indicate the desirability of future toxicologic and epidemiologic research.

The performance of rats in a T-maze and a Y-maze after exposure to sarin or sarin plus oximes was somewhat affected, but the performance of some of the control animals was also lower than expected in some of the studies with oximes. Many of the effects were reversed by 3 months.

Some studies have looked at expression of astroglial markers 1 and 2 hours and 1, 3, and 7 days after treatment. Glial fibrillary acidic protein and vimentin were increased in the areas of the brain studied (cortex, midbrain, cerebellum, brainstem, and spinal cord), and vimentin induction occurred sooner. Some effects on expression of both could still be detected 7 days after treatment.

Immunotoxicity

Both in vivo and in vitro immune effects have been seen, but they are not consistent and they depend on the cell types studied. Recent studies have investigated persistent effects of sarin on the immune system. Modest and inconsistent effects on lymphocyte proliferation and production of *N*-oxides were seen in rats 3 months after a single or repeated (three times in 1 week) 1-hour inhalation-chamber exposure.

Genotoxicity and Carcinogenicity

In general, genotoxicity studies (of mutagenesis, chromosomal damage, unscheduled DNA synthesis, or sister chromatid exchange) are negative. In a subchronic (90-day) toxicologic study of sarin (three different doses that produced profound inhibition of AChE and some deaths), one of two formulations of sarin was associated with one neoplastic lesion, a lymphoma, in one male in the high-dose group.

No chronic animal studies have been conducted to determine the carcinogenic effects of exposure to sarin.

Genetic Susceptibility

One of the mechanisms of sarin inactivation is hydrolysis with the enzyme paraoxonase (PON1), an esterase synthesized and secreted by the liver. The human PON1 gene has polymorphisms that affect serum PON1 activity and therefore might significantly alter susceptibility to the toxicity of sarin. The relationship between illness in Gulf War veterans and PON1 genotype and serum activity has been investigated. The results of one study suggested that low PON1 activity due to the polymorphism might be a risk factor for illness in Gulf War veterans, but another study did not find any differences in PON1 activity between symptomatic and asymptomatic Gulf War veterans.

HUMAN HEALTH OUTCOME DATA

Four populations have been studied in large epidemiologic studies after exposure to sarin: military volunteers who were exposed several decades ago to nonlethal doses of sarin and other chemical-warfare agents, industrial workers with documented acute exposure to sarin, victims of the sarin terrorist attacks in Matsumoto City in 1994 and Tokyo in 1995, and Gulf War veterans. Studies of Gulf War veterans include studies of veterans potentially exposed to sarin after demolition of rockets at Khamisiyah and a number of studies on Gulf War veterans that evaluate the relationship between symptoms and possible exposures on

the basis of a self-reporting questionnaire, including possible exposures to sarin or cyclosarin.

Neurologic Effects

A number of studies have evaluated the possible relationship between exposure to sarin or cyclosarin and neurologic effects in humans. Those outcomes have been the focus of the largest number of studies because of the neurotoxic actions of the chemicals.

Studies of British and US military servicemen who volunteered for an experimental study of the health effects of low-dose exposure to sarin and other chemical-warfare agents did not demonstrate any long-term health effects of exposure to cholinesterase inhibitors. In both the US and British studies, some subjects experienced the acute cholinergic syndrome.

Studies have followed the health effects in people who exhibited the acute cholinergic syndrome after terrorist attacks in Matsumoto and Tokyo, Japan. Three years after the Matsumoto attack, fatigue, headache, and the visual disturbances asthenopia, blurred vision, and narrowing of visual field were more common among people who reported signs of the acute cholinergic syndrome than among those who lived near the sarin release site who did not have signs of the syndrome. An English-language abstract also showed visual-field constriction and abnormal EEG 45 months after the attacks. Some 6–8 months after the Tokyo attack, symptom-free survivors of intermediate to high exposures were impaired on only one of nine neurobehavioral tests, and significant changes on some EEG results and postural sway tests were seen in females. Three years after the Tokyo attack, a dose-effect relationship was found in previously poisoned people on a measure of memory performance (the backward digit span test), and tapping interval for the dominant hand and stabilometry measures with eyes open were affected in exposed people. An uncontrolled study of patients after the Tokyo attack showed ocular effects (tiredness of eyes, dim vision, and difficulty in focusing), tiredness, fatigue, stiff muscles, and headache up to 5 years after the attack.

Studies have been conducted on US troops who were potentially exposed to sarin after munitions demolition at Khamisiyah. There are no reports that any troops had signs of the acute cholinergic syndrome. Studies of veterans showed no differences between troops who were and who were not present at Khamisiyah. However, when they were divided into those who reported that they had or had not witnessed the explosion and were questioned about symptoms present 8 years after the explosion, those who reported witnessing the explosion were more likely to have self-reported changes in memory, difficulty in sleeping, persistent fatigue, and depression.

In addition to the studies of troops potentially exposed to sarin at Khamisiyah, a number of studies have been conducted on cohorts from the Gulf War that

included analyses of possible indicators of sarin exposure on questionnaires. Self-reports indicating exposure to "chemical-warfare agents" were associated with various neurologic findings in a number of studies. The outcomes in the different studies were cognitive dysfunction, depression, and fibromyalgia; major depression and anxiety; a syndrome termed "confusion–ataxia" (problems with thinking, disorientation, balance disturbances, vertigo, and impotence); mood, memory, and cognitive deficits (profile of mood states, tension and confusion scales, three tests of recall memory, and the WMS-R backward digit span test of memory); and musculoskeletal, neurologic, neuropsychologic, and psychologic symptoms.

Some studies have not shown such effects. In a study of Danish Gulf War veterans, all of whom were involved in peacekeeping or humanitarian roles after the end of the war, self-reported exposure to "nerve gas" was not significantly associated with the neuropsychologic symptoms in the Gulf War cohort. Exposure of those troops to sarin, however, was unlikely. One study of US troops reported no association with symptoms of cognitive dysfunction, chronic fatigue, and fibromyalgia.

Posttraumatic stress disorder (PTSD) has been seen in survivors of the Matsumoto and Tokyo sarin terrorist attacks and in British veterans who reported either wearing "nuclear, biological, and chemical warfare suits", hearing chemical alarms, or having a "chemical/nerve gas attack". Other studies, however, found no relationship between PTSD and "wearing chemical protective gear or hearing alarms sounding", and PTSD was not more common among Khamisiyah-exposed than nonexposed Gulf War veterans. It is not known whether PTSD would be caused by the chemical itself or by the traumatic event.

Cardiovascular Effects

There have been some reports of persistent cardiovascular effects following the sarin attacks in Japan—sudden palpitation and electrocardiographic (ECG) changes—but other studies report that no ECG changes were evident in recovered victims 6–8 months after the Tokyo attack. A study of military personnel deployed during the time of Khamisiyah found one (cardiac dysrhythmias) of 10 specific self-reported physician cardiac diagnoses to be more frequent in the exposed versus nonexposed people. Other studies of veterans showed various cardiovascular effects, but only for deployed versus nondeployed veterans, with no analysis for exposure to sarin.

Other Health Effects

The presence of multisymptom illness, Gulf War illness, or unexplained illness and the relationship of any of them to possible indicators of exposure to chemical-warfare agents has been studied in Gulf War veterans. A case of Gulf

War illness was associated with "use of gas masks". Another study found that responding yes to "thought biological or chemical weapons were being used" was associated with meeting the criteria for a severe or mild to moderate case of multisymptom illness; and another found an association between high frequency of "placement on formal alert for chemical and biological warfare" and mild to moderate or severe multisymptom illness. The prevalence of multiple chemical sensitivity was also associated with hearing chemical alarms and self-reports of having a chemical or nerve-gas attack; and chronic fatigue syndrome was associated with hearing chemical alarms in a study of British soldiers. In all those studies, the exposure assessment is not a reliable indicator of actual sarin or cyclosarin exposures.

HEALTH OUTCOME CONCLUSIONS

The present committee weighed the strengths and limitations of all the epidemiologic evidence reviewed in this report and in *GW1* and reached its conclusions by interpreting the new evidence in the context of the entire body of literature. It assigned each health outcome being considered to one of five categories on the basis of that evidence. The definitions of the categories and the criteria for assigning particular health outcomes to each category are described in Table ES-1; the health outcomes assigned to each category are also listed in the table.

It should be noted that the committee was charged with reviewing the scientific data, not with making recommendations regarding VA policy; therefore, conclusions are not intended to imply or suggest policy decisions. Furthermore, the conclusions are related to associations between exposure to chemicals and health outcomes in human populations, not to the likelihood that any one person's health problem is associated with or caused by exposure to sarin or cyclosarin.

The committee's conclusions are presented in Box ES-1.

TABLE ES-1 Summary of Findings Regarding the Association Between Specific Health Outcomes and Exposure to Sarin or Cyclosarin

Sufficient Evidence of a Causal Relationship

Evidence from available studies is sufficient to conclude that a causal relationship exists between exposure to a specific agent and a specific health outcome in humans, and the evidence is supported by experimental data. The evidence fulfills the guidelines for sufficient evidence of an association (below) and satisfies several of the guidelines used to assess causality: strength of association, dose–response relationship, consistency of association, biologic plausibility, and a temporal relationship.

- **Exposure to sarin and a dose-dependent acute cholinergic syndrome that is evident seconds to hours subsequent to sarin exposure and resolves in days to months**

Sufficient Evidence of an Association

Evidence from available studies is sufficient to conclude that there is a positive association. A consistent positive association has been observed between exposure to a specific agent and a specific health outcome in human studies in which chance[1] and bias, including confounding, could be ruled out with reasonable confidence. For example, several high-quality studies report consistent positive associations, and the studies are sufficiently free of bias, including adequate control for confounding.

Limited/Suggestive Evidence of an Association

Evidence from available studies suggests an association between exposure to a specific agent and a specific health outcome in human studies, but the body of evidence is limited by the inability to rule out chance and bias, including confounding, with confidence. For example, at least one high-quality[2] study reports a positive association that is sufficiently free of bias, including adequate control for confounding. Other corroborating studies provide support for the association, but they are not sufficiently free of bias, including confounding. Alternatively, several studies of less quality show consistent positive associations, and the results are probably not[3] due to bias, including confounding.

- **Exposure to sarin at doses sufficient to cause acute cholinergic signs and symptoms and a variety of subsequent long-term neurological effects[4]**

Inadequate/Insufficient Evidence to Determine Whether an Association Exists

Evidence from available studies is of insufficient quantity, quality, or consistency to permit a conclusion regarding the existence of an association between exposure to a specific agent and a specific health outcome in humans.

- **Exposure to sarin at low doses insufficient to cause acute cholinergic signs and symptoms and subsequent long-term adverse neurological health effects**
- **Exposure to sarin and subsequent long-term cardiovascular effects**

Continued

TABLE ES-1 Continued

Limited/Suggestive Evidence of No Association

Evidence from well-conducted studies is consistent in not showing a positive association between exposure to a specific agent and a specific health outcome after exposure of any magnitude. A conclusion of no association is inevitably limited to the conditions, magnitudes of exposure, and length of observation in the available studies. The possibility of a very small increase in risk after exposure studied cannot be excluded.

[1]Chance refers to sampling variability.

[2]Factors used to characterize high-quality studies include the statistical stability of the associations, whether dose–response or other trends were demonstrated, whether or not the association was among numerous comparisons that were made, and the quality of the assessments of exposure and outcome. Specifically, the quality of exposure assessment refers to specificity and sensitivity in relation to the association of interest. For instance, for insecticides, studies assessing specific insecticides (such as chlorpyrifos) have more specificity than those assessing classes of insecticides (such as organophosphorus), which in turn are more specific than those assessing pesticides more generally. With respect to sensitivity, studies are judged by the instruments used to measure exposure. Biologic monitoring data are theoretically the most preferable but are almost never obtainable in the context of a nonpersistent chemical and a disease with long latency, like cancer. Other kinds of efforts can obtain sensitive measures of exposure, such as use of occupational or environmental monitoring data, use of more extensive industrial hygiene assessments, use of interview techniques that help to minimize recall bias (for example, photos of products and home and workplace walkthroughs). Similarly, there are questions about quality of outcome assessment—whether an outcome has been verified by a medical diagnosis in a consistent fashion.

[3]Factors used to make this judgment include the data on the relationship between potential confounders and related health end points in a given study, information on subject selection, and classification of exposure.

[4]See Chapters 3 and 4, and Box ES-1 for further details.

BOX ES-1
Health Outcome Conclusions

Short-Term Neurological Effects Following Acute Exposures

There is sufficient evidence of a causal relationship between exposure to sarin and a dose-dependent acute cholinergic syndrome that is evident seconds to hours subsequent to sarin exposure and resolves in days to months.

The acute cholinergic syndrome has been recognized for decades. The syndrome, as well as cholinergic signs and symptoms, is evident seconds to hours after exposure and usually resolves in days to months.

Long-Term Neurological Effects Following Acute Exposures

There is limited/suggestive evidence of an association between exposure to sarin at doses sufficient to cause acute cholinergic signs and symptoms and a variety of subsequent long-term neurological effects.

Many health effects are reported in the literature to persist after sarin exposure: fatigue, headache, visual disturbances (asthenopia, blurred vision, and narrowing of the visual field), asthenia, shoulder stiffness, and symptoms of PTSD. Sarin exposure has been followed by abnormal test results, of unknown clinical significance, on the digit symbol test of psychomotor performance, EEG records of sleep, event-related potential, visual evoked potential, and computerized posturography.

Persistent Neurological Effects Following Low-Level Exposures

There is inadequate/insufficient evidence to determine whether an association does or does not exist between exposure to sarin at low doses insufficient to cause acute cholinergic signs and symptoms and subsequent long-term adverse neurological health effects.

In the absence of carefully designed human studies expressly of sarin or cyclosarin's long-term health effects at doses that do not produce acute signs and symptoms, the committee concludes that the data remain inadequate or insufficient to determine whether such effects exist.

Persistent Cardiovascular Effects Following Low-Level Exposures

There is inadequate/insufficient evidence to determine whether an association does or does not exist between exposure to sarin and subsequent long-term cardiovascular effects.

Studies of persistent cardiovascular effects after sarin exposure have been inconsistent. Therefore, the committee concluded that the data are inadequate or insufficient to determine whether an association exists.

1

Introduction

The Gulf War in 1990–1991 was considered a brief and successful military operation, with few injuries or deaths of US troops. The war began in August 1990, and the last US ground troops returned home by June 1991. Although most Gulf War veterans resumed their normal activities, many soon began reporting a variety of nonexplained health problems that they attributed to their participation in the Gulf War, including chronic fatigue, muscle and joint pain, loss of concentration, forgetfulness, headache, and rash.

In response to concerns about the veterans' health problems, the Department of Veterans Affairs (VA) requested that the Institute of Medicine (IOM) review the scientific and medical literature on the long-term adverse health effects of agents to which the Gulf War veterans may have been exposed. In 1998, IOM and the VA entered into a contract for a series of studies that would provide conclusions about the strength of the association between exposure to the agents of concern and health outcomes as observed in the epidemiologic literature.

Congress, also responding to the growing concerns of ill veterans, passed legislation in 1998 for a study similar to that previously requested by the VA (the Persian Gulf War Veterans Act of 1998, PL 105–277, and the Veterans Programs Enhancement Act of 1998, PL 105–368). The legislation directed the secretary of veterans affairs to enter into an agreement with IOM to review the literature on 33 agents related to service in the Gulf War and to assess the strength of associations between exposure to those agents and long-term adverse health effects as noted in the published literature. The legislation directs the secretary to consider the IOM conclusions when making decisions about compensation.

In response to those requests, IOM has convened three committees to evalu-

ate the health effects of various chemicals used in the Gulf War. The first evaluated the health effects of depleted uranium, pyridostigmine bromide, sarin and cyclosarin, and vaccines (anthrax and botulinum toxoid) and produced *Gulf War and Health, Volume 1*, hereafter referred to as *GW1* (IOM, 2000a). The second reviewed the health effects of solvents and pesticides and produced *Gulf War and Health, Volume 2,* hereafter referred to as *GW2* (IOM, 2003a). The third is reviewing the health effects of the combustion products of oil-well fires, fuels, and synthetic compounds potentially used as propellants for Scud missiles.

Sarin (GB; *o*-isopropyl methylphosphonofluoridate) and cyclosarin (GF; cyclohexyl methylphosphonofluoridate) are highly toxic organophosphorus (OP) nerve agents produced for chemical-warfare. They were first synthesized in Germany before and during World War II (Somani, 1992), and the first military use of sarin occurred in the Iran–Iraq conflict in the 1980s (Brown and Brix, 1998). Sarin and cyclosarin exert many of their effects by irreversibly binding to and inactivating acetylcholinesterase (AChE), the enzyme responsible for metabolizing the neurotransmitter acetylcholine (ACh). The inactivation of AChE results in an increase in ACh at cholinergic synapses (Gunderson et al., 1992) and overstimulation of muscles and nerves. After sufficient exposure to sarin or other OP nerve agents, that overstimulation causes what has been termed the acute cholinergic syndrome (see Chapter 2).

Because of continued concerns of veterans, especially in light of recent toxicologic studies of low-dose exposure to sarin, the VA requested that IOM update its evaluation of the health effects of sarin. In response to that request, IOM convened the Committee on Gulf War and Health: Updated Literature Review of Sarin, which is responsible for the present report. The committee consists of several members of the committee responsible for *GW*2 (IOM, 2003a), which evaluated the health effects of pesticides and solvents used in the Gulf War.

POTENTIAL US TROOP EXPOSURE

During a cease-fire period in March 1991, troops from the US 37th and 307th engineering battalions destroyed enemy munitions throughout the occupied areas of southern Iraq (PAC, 1996). A large storage complex at Khamisiyah, Iraq, which contained more than 100 bunkers, was destroyed. Two sites in the complex—one of the bunkers and another site called the "pit"—contained stacks of 122-mm rockets loaded with sarin and cyclosarin (Committee on Veterans Affairs, 1998). According to the most recent estimates, 371 kg of sarin and cyclosarin combined was released (Winkenwerder, 2002). US troops performing demolitions were unaware of the presence of nerve agents because their detectors, being sensitive only to lethal or near-lethal concentrations of nerve agents (CDC, 1999), did not sound any alarms before demolition. It was not until October 1991 that inspectors from the United Nations Special Commission (UNSCOM) confirmed

the presence of a mixture of sarin and cyclosarin at Khamisiyah (Committee on Veterans Affairs, 1998).

No air monitoring was conducted at the time of the Khamisiyah demolition. At the request of the Presidential Advisory Committee (PAC), the Central Intelligence Agency (CIA) and the Department of Defense (DOD) used models to estimate ground-level concentrations of sarin and cyclosarin as a function of distance and direction from the detonation sites and then to estimate the extent of potential exposure of US military personnel to the nerve agents (PAC, 1996). The models produced a series of geographic maps of the Khamisiyah area that overlay known troop unit locations with the projected path of the sarin–cyclosarin plume. Initially, however, because of the complexity of the modeling that needed to be done, CIA–DOD estimated that any noticeable effects of sarin and cyclosarin would possibly have been seen within 25 km of the demolition site. The CIA–DOD report estimated, on the basis of troop locations, that about 10,000 US troops had been within 25 km and thus might have been exposed to sarin or cyclosarin over a period of hours (CIA–DOD, 1997). Given the uncertainties in that estimate, CIA–DOD doubled the distance and, again on the basis of unit locations, estimated that roughly 20,000 troops were within 50 km. In 1997, DOD mailed a survey to the 20,000 troops who were within 50 km of Khamisiyah; of the 7,400 respondents, more than 99% reported no acute effects that could be correlated with exposure to sarin or cyclosarin (CIA–DOD, 1997). The survey was attached to a letter from the secretary of veterans affairs indicating that chemical weapons had been present at Khamisiyah at the time of the demolitions. The letter also urged survey recipients to call the Gulf Incident Hotline with any additional information about the Khamisiyah incident or to report illnesses they attributed to their service in the Gulf War.

The CIA–DOD models integrated four components:

- UNSCOM reporting and intelligence summaries of the amount, purity, and type of chemical-warfare agents stored at Khamisiyah.
- Results of experiments[1] performed later at Dugway Proving Ground to simulate the demolition at Khamisiyah and thus estimate the amount of sarin and cyclosarin released, the release rate, and the type of release (instantaneous, continuous, or fly-out).
- A combination of dispersion models that incorporated meteorologic conditions at the time (including wind direction) to simulate the transport and diffusion of the plume so that agent concentrations downwind could be estimated.

[1]These experiments used a substitute chemical (triethyl phosphate) to simulate chemical-warfare agent and measured agent release concentrations after replicating the rockets in the pit, terrain, original warhead design, stacking of rockets, and other relevant information.

• Unit location information to determine the position of troops in relation to the plume's path (CIA–DOD, 1997).

Potential exposure was categorized as a "first-noticeable-effects" level and a "general-population" level. At the first-noticeable-effects level, for which the lower limit was set at 1 mg-min/m^3, the estimated exposure would be high enough to cause watery eyes, runny nose, tightness of chest, sweating, muscle twitching, or other early signs of exposure to OP compounds. The general-population level, for which the upper limit was set at 0.01296 mg-min/m^3, was the "dosage below which the general population, including children and older people, could be expected to remain 72 hours with no effects". Between those two was the "area of low-level exposure" (CIA–DOD, 1997). The models indicated that the plume of air concentrations that were the first-noticeable-effects levels would have dispersed to below 1 mg-min/m^3 within 3 days of the demolition. The plume of air concentrations in the low-level range dispersed to be in the general-population level within 5 days of the demolition. Taking the potential first-noticeable-effects exposures and the potential low-level exposures into account, and eliminating the counting of the same troops on multiple days, CIA–DOD estimated that nearly 99,000 troops might have been exposed to sarin or cyclosarin above the general-population level over the course of 4 days after the demolition of the pit at Khamisiyah. Those CIA–DOD findings were challenged in a US Senate report (Committee on Veterans' Affairs, 1998). The Senate report took issue with the methodology, especially the reconstruction of the pit site, with the nature of the demolition, and with the number of exposed troops.

At the request of the Senate Committee on Veterans' Affairs, the Air Force Technical Applications Center (AFTAC) prepared another exposure model. The AFTAC report summary—the only portion of the report made public—indicates that AFTAC used models different from those used by CIA–DOD to simulate atmospheric chemistry (Committee on Veterans' Affairs, 1998). The report indicated additional geographic areas of low-level exposure not modeled by CIA–DOD. Neither the AFTAC nor the CIA–DOD report described above appears to have undergone independent peer review.

A second CIA–DOD model, revised from the first and peer-reviewed, was completed in 2000 (Rostker, 2000), and a final report was released in 2002 (Winkenwerder, 2002). The second CIA–DOD model differed from the first in that it incorporated updated unit location and personnel data, revised meteorologic models, reduced estimates of nerve-agent release, combined toxicity of sarin and cyclosarin (the first model used only sarin), and adjusted the general population level to account for a briefer duration of troops' potential exposure. Troops were considered exposed at an exposure rate of 0.0432 mg-min/m^3 for sarin and 0.0144 mg-min/m^3 for cyclosarin.

Neither of the models found any troops to have been exposed above first-

noticeable-effects levels at which concentrations would have been high enough to induce a particular type of chemical alarm to sound and visible signs of the acute cholinergic syndrome among troops. No medical reports by the US Army Medical Corps at the time of the release were consistent with signs and symptoms of acute exposure to sarin (PAC, 1996). That is in accordance with the results of the survey completed by 7,400 troops within 50 km of Khamisiyah: no reports of cholinergic effects (CIA–DOD, 1997).

Two other storage sites in central Iraq, Muhammadiyat and Al Muthanna, sustained damage from air attacks during the Gulf War. Munitions containing 2.9 metric tons of sarin–cyclosarin and 1.5 metric tons of mustard gas were damaged at Muhammadiyat, and munitions containing 16.8 metric tons of sarin–cyclosarin were damaged at Al Muthanna (PAC, 1996). Atmospheric modeling by CIA–DOD determined that the nearest US personnel—400 km away—were outside the range of contamination (PAC, 1996).

In summary, exposure models indicate that sarin–cyclosarin release occurred in March 1991 as a result of US demolition of a storage depot in Khamisiyah, Iraq. Exposure models indicate that the degree of exposure of US troops in the path of the sarin–cyclosarin plume was low. Two other storage sites in central Iraq that contained sarin, cyclosarin, and mustard gas were damaged in air attacks, but modeling indicates that all US troops were outside the range of contamination from those sites. All that information is consistent with the absence of reports of symptoms of an acute cholinergic syndrome by medical personnel or veterans.

PREVIOUS IOM CONCLUSIONS ON SARIN AND CYCLOSARIN

GW1 (IOM, 2000a) evaluated the health effects of the chemical-warfare agents sarin and cyclosarin. The *GW1* committee was unable to formulate conclusions about cyclosarin, because of the paucity of toxicologic and human studies. After a review of the literature on sarin, the committee reached three conclusions, as follows:

> There is sufficient evidence of a causal relationship between exposure to sarin and a dose-dependent acute cholinergic syndrome that is evident seconds to hours subsequent to sarin exposure and resolves in days to months.

The acute cholinergic syndrome has been recognized for decades and has been documented in human studies summarized in this chapter. The syndrome is evident seconds to hours after exposure and usually resolves in days to months. The syndrome is produced by sarin's irreversible inhibition of AChE. Inactivation of the enzyme leads to the accumulation of ACh at cholinergic synapses. Excess ACh results in widespread overstimulation of muscles and nerves. At high doses, convulsions and death can occur.

> There is limited/suggestive evidence of an association between exposure to sarin at doses sufficient to cause acute cholinergic signs and symptoms and subsequent long-term health effects.

Many health effects are reported to persist after sarin exposure: fatigue, headache, visual disturbances (asthenopia, blurred vision, and narrowing of the visual field), asthenia, shoulder stiffness, symptoms of posttraumatic stress disorder, and abnormal results (of unknown clinical significance) on the digit symbol test of psychomotor performance, electroencephalographic records of sleep, event-related potential, visual evoked potential, and computerized posturography.

Those conclusions were based on retrospective studies of three exposed populations in which the cholinergic signs and symptoms were documented as acute effects of exposure. The findings in those studies were based on comparisons with control populations—one of industrial workers accidentally exposed to sarin in the United States and two of civilians exposed during terrorism episodes in Japan. The health effects listed above were documented at least 6 months after sarin exposure, and some persisted for up to 3 years, depending on the study. Whether the health effects noted above persist beyond 3 years had not been studied.

> There is inadequate/insufficient evidence to determine whether an association does or does not exist between exposure to sarin at low doses insufficient to cause acute cholinergic signs and symptoms and subsequent long-term adverse health effects.

On the basis of findings in a study of nonhuman primates and in studies of humans exposed to OP insecticides, it is reasonable to hypothesize the occurrence of long-term adverse health effects of exposure to low concentrations of sarin. Studies of low exposure of workers have found that OP insecticides are associated with a higher prevalence of neurologic or psychiatric symptoms. However, no well-controlled human studies have looked expressly at sarin's long-term health effects at doses that do not produce acute signs and symptoms.

CHARGE TO THE PRESENT COMMITTEE

The present committee was charged to review the peer-reviewed literature published since earlier IOM reports on health effects associated with exposure to sarin and related compounds, including relevant epidemiologic studies. With regard to the toxicologic literature, the committee used review articles to obtain and present a broad overview of the toxicology of sarin and cyclosarin and to assess biologic plausibility with respect to the compounds in question and health effects; individual toxicologic research papers were evaluated as warranted. The committee based determinations on the strength of the evidence of associations between the compounds and human health effects. If published, peer-reviewed

information was available on the magnitude of sarin and cyclosarin exposure of Gulf War veterans, the committee addressed the potential health risks posed to the veterans. The committee also considered other relevant issues, such as exposure to multiple chemicals and genetic susceptibilities. The committee's review included recommendations for additional scientific studies to resolve continued scientific uncertainty as warranted.

The committee was not charged with determining whether a unique Gulf War syndrome exists, nor was it to make judgments regarding magnitudes of exposure of veterans to the putative agents. Moreover, the committee was not charged to focus on broader issues, such as the potential costs of compensation for veterans or policies regarding such compensation. Those decisions remain the responsibility of the secretary of veterans affairs. This report does, however, provide an assessment of the scientific evidence regarding health effects that may be associated with exposures to specific agents that were present in the Gulf War. The secretary may consider those health effects as the VA develops a compensation program for Gulf War veterans.

APPROACH TO THE CHARGE

The committee's first step was to identify the literature that it would review. The search was conducted by using the names of sarin and cyclosarin and their synonyms. The search resulted in the retrieval of about 250 titles published from the time of preparation of *GW1* (IOM, 2000a) to November 2003. Those titles and abstracts were reviewed to determine their relevance to the committee's charge; potentially relevant studies were retrieved and evaluated. The literature was also searched for epidemiologic studies on OP compounds published since the last literature search for the preparation of *GW2* (March, 1999), and such studies were reviewed. The committee used only published, peer-reviewed titles to draw its conclusions. Although the process of peer review by fellow professionals enhances the likelihood that a study has reached valid conclusions, it does not guarantee it. Accordingly, committee members read each study and considered its relevance and quality. The committee did not collect original data or perform any secondary data analysis.

A great deal of research, including much epidemiologic work, has been conducted on the health effects of other OP compounds that are used as insecticides. As discussed in Chapter 2, most of the acute effects of sarin are thought to be mediated by inhibition of AChE, a common mechanism among OP compounds that leads to the acute cholinergic syndrome. Because of that common mechanism of action, it is possible that studies of those insecticides could provide some insight into potential health effects of sarin and cyclosarin. The mechanisms underlying any possible effects of low doses of the insecticides and nerve agents, however, are not yet understood. Therefore, the committee did not base its conclusions of the results of studies of the insecticides. Despite not using the

OP insecticide data in its conclusion, the committee reviewed the OP epidemiology literature. The committee responsible for *GW2* (IOM, 2003a) reviewed the literature on OP compounds. The present committee reviewed relevant epidemiology studies published since the preparation of that report.

Animal studies had a small role in the committee's assessment of association between putative agents and health outcomes. As with previous committees, this committee used animal data for making assessments of biologic plausibility in support of the epidemiologic data rather than as part of the weight of evidence to determine the likelihood that an exposure to a specific agent might cause a long-term outcome.

The committee classified the evidence of an association between exposure to sarin and cyclosarin and a specific health outcome into five categories (Box 1-1). The categories closely resemble those used by previous committees that evaluated the effects of chemicals related to the Gulf War (IOM, 2000a, 2003a) and those used by several IOM committees that have evaluated vaccine safety (IOM, 1991, 1994a), herbicides used in Vietnam (IOM, 1994b, 1996, 1999, 2001, 2003b), and indoor pollutants related to asthma (IOM, 2000b). The committee's conclusions, presented in Chapter 4, represent its collective judgment.

The committee endeavored to express its judgment as clearly and precisely as the available data allowed, and it used the established categories of association from previous IOM studies because they have gained wide acceptance over more

BOX 1-1
Categories of Evidence

Sufficient Evidence of a Causal Relationship

Evidence from available studies is sufficient to conclude that a causal relationship exists between exposure to a specific agent and a specific health outcome in humans, and the evidence is supported by experimental data. The evidence fulfills the guidelines for sufficient evidence of an association (below) and satisfies several of the guidelines used to assess causality: strength of association, dose–response relationship, consistency of association, biologic plausibility, and a temporal relationship.

Sufficient Evidence of an Association

Evidence from available studies is sufficient to conclude that there is a positive association. A consistent positive association has been observed between exposure to a specific agent and a specific health outcome in human studies in which chance[1] and bias, including confounding, could be ruled out with reasonable confidence. For example, several high-quality studies report consistent positive associations, and the studies are sufficiently free of bias, including adequate control for confounding.

Limited/Suggestive Evidence of an Association

Evidence from available studies suggests an association between exposure to a specific agent and a specific health outcome in human studies, but the body of evidence is limited by the inability to rule out chance and bias, including confounding, with confidence. For example, at least one high-quality[2] study reports a positive association that is sufficiently free of bias, including adequate control for confounding. Other corroborating studies provide support for the association, but they are not sufficiently free of bias, including confounding. Alternatively, several studies of less quality show consistent positive associations, and the results are probably not[3] due to bias, including confounding.

Inadequate/Insufficient Evidence to Determine Whether an Association Exists

Evidence from available studies is of insufficient quantity, quality, or consistency to permit a conclusion regarding the existence of an association between exposure to a specific agent and a specific health outcome in humans.

Limited/Suggestive Evidence of No Association

Evidence from well-conducted studies is consistent in not showing a positive association between exposure to a specific agent and a specific health outcome after exposure of any magnitude. A conclusion of no association is inevitably limited to the conditions, magnitudes of exposure, and length of observation in the available studies. The possibility of a very small increase in risk after exposure studied cannot be excluded.

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[1]Chance refers to sampling variability.

[2]Factors used to characterize high-quality studies include the statistical stability of an association, whether or not dose–response or other trends were demonstrated, whether or not the association was among numerous comparisons that were made, and the quality of the assessments of exposure and outcome. Specifically, the quality of exposure assessment refers to specificity and sensitivity in relation to the association of interest. For instance, for insecticides, studies assessing specific insecticides (such as chlorpyrifos) have more specificity than those assessing classes of insecticides (such as organophosphorus), which in turn are more specific than those assessing pesticides more generally. With respect to sensitivity, studies are judged by the instruments used to measure exposure. Biologic monitoring data are theoretically the most preferable but are almost never obtainable in the context of a nonpersistent chemical and a disease with long latency, such as cancer. Other kinds of efforts can obtain sensitive measures of exposure, such as use of occupational or environmental monitoring data, use of more extensive industrial hygiene assessments, use of interview techniques that help to minimize recall bias (for example, photos of products and home and workplace walkthroughs). Similarly, there are questions about quality of outcome assessment—whether an outcome has been verified by a medical diagnosis in a consistent fashion.

[3]Factors used to make this judgment include data on the relationship between potential confounders and related health end points in a given study, information on subject selection, and classification of exposure.

than a decade by Congress, government agencies, researchers, and veterans groups. The five categories describe different levels of association and sound a recurring theme: the validity of an association is likely to vary with the extent to which the authors reduced common sources of error in drawing inferences—chance variation, bias, and confounding. Accordingly, the criteria for each category express a degree of confidence based on the extent to which sources of error were reduced.

As the committee began its evaluation, neither the existence nor the absence of an association was presumed. Rather, the committee weighed the strengths and weaknesses of the available evidence, including studies reviewed in *GW1* and recent studies, to reach conclusions. It should be noted that although *causation* and *association* are often used interchangeably, they are not the same; an association can indicate an increase in risk even if exposure to the putative agent is not the sole or even primary cause.

Epidemiologic studies can establish statistical associations between exposure to specific agents and health effects, and associations are generally estimated by using relative risks or odds ratios. To conclude that an association exists, it is necessary for exposure to an agent to be followed by the health outcome more frequently than it would be expected to by chance alone. Furthermore, it is almost always necessary to find that the effect occurs consistently in several studies. Epidemiologists seldom consider a single study sufficient to establish an association; rather, it is desirable to replicate the findings in other studies to draw conclusions about the association. Results of separate studies are sometimes conflicting. It is sometimes possible to attribute discordant study results to such characteristics as soundness of study design, quality of execution, and the influence of different forms of bias. Studies that result in a statistically precise measure of association suggest that the observed result was unlikely to be due to chance. When the measure of association does not show a statistically precise effect, it is important to consider the size of the sample and whether the study had the power to detect an effect of a given size.

Epidemiology concerns itself with the study of the determinants, frequency, and distribution of disease in human populations. A focus on populations distinguishes epidemiology from medical disciplines that focus on the individual. Epidemiologic studies examine the relationship between exposures to agents of interest in a studied population and the development of health outcomes, so they can be used to generate hypotheses for study or to test hypotheses posed by investigators.

Epidemiology study designs differ in their ability to provide valid estimates of an association (Ellwood, 1998). Randomized controlled trials yield the most robust type of evidence; cohort or case–control studies are more susceptible to bias. Cross-sectional studies generally provide a lower level of evidence than cohort and case–control studies. Determining whether a given statistical association rises to the level of causation requires inference (Hill, 1965). As discussed by

the International Agency for Research on Cancer in the preamble of its monographs evaluating cancer risks (for example, IARC, 2004), a strong association in an epidemiology study, an association being seen in a number of different studies, an increase risk of disease with increasing amount of exposure or a decline in risk of disease after cessation of exposure, and specificity of an effect all strengthen the likelihood that an association seen in epidemiology study is a causal effect. Inferences from epidemiology studies, however, are often limited to population associations in many cases because of a lack of exposure information. Exposures are not controlled in epidemiology studies and, in some cases, there is large uncertainty in the assessment of the exposure. That is especially the case for the available epidemiology studies of sarin. To assess explanations other than causality, one must bring together evidence from different studies and apply well-established criteria (which have been refined over more than a century) (Evans, 1976; Hill, 1965; Susser, 1973, 1977, 1988, 1991; Wegman et al., 1997). The strengths and limitations of the various epidemiology designs, the issues to be considered when assessing epidemiology studies, and the outcomes measured in the studies are discussed in detail in Chapter 2 of *GW2* (IOM, 2003a).

By examining numerous epidemiologic studies, the committee addressed the question, "Does the available evidence support a causal relationship or an association between exposure to a specific agent and a health outcome?" An association between a specific agent and a specific health outcome does not mean that exposure to the agent invariably results in the health outcome or that all cases of the health outcome result from exposure to the agent. Such complete correspondence between agent and disease is the exception in large populations (IOM, 1994b). The committee evaluated the data and based its conclusions on the strength and coherence of the data in the selected studies.

ORGANIZATION OF REPORT

The remainder of this report is organized into three chapters and an appendix. Chapter 2 reviews the relevant animal and in vitro toxicology data to provide background on the mechanism of action of sarin and cyclosarin and to permit evaluation of the biologic plausibility of any effects seen in the epidemiologic literature. Chapter 3 reviews relevant epidemiologic studies published since *GW1* to provide the basis of the committee's conclusions on the health effects of sarin and cyclosarin. The committee's overall conclusions on the health effects of sarin and cyclosarin, with summaries of the studies and the rationale that lead to those conclusions, are presented in Chapter 4. As discussed above, sarin and cyclosarin are OP compounds and therefore have some mechanisms of action in common with OP insecticides, which have been more thoroughly studied than the chemical-warfare agents. In light of those similarities, in Appendix A the committee has summarized the conclusions on OP insecticides

from *GW2* and reviewed epidemiologic data on those insecticides published since *GW2* (IOM, 2003a).

REFERENCES

Brown MA, Brix KA. 1998. Review of health consequences from high-, intermediate- and low-level exposure to organophosphorus nerve agents. *Journal of Applied Toxicology* 18(6):393–408.

CDC (Centers for Disease Control and Prevention). 1999. *Background Document on Gulf War-Related Research for the Health Impact of Chemical Exposures During the Gulf War: A Research Planning Conference*. Atlanta, GA: CDC.

CIA–DOD (Central Intelligence Agency and Department of Defense). 1997. *Modeling the Chemical Warfare Agent Release at the Khamisiyah Pit*. Washington, DC: CIA–DOD.

Committee on Veterans Affairs (US Senate). 1998. *Report of the Special Investigation Unit on Gulf War Illnesses*. 105th Congress, 2nd session. Washington, DC: US Government Printing Office. S.PRT 105-139.

Ellwood JM. 1998. *Critical Appraisal of Epidemiological Studies and Clinical Trials*. 2nd ed. Oxford, UK: Oxford University Press.

Evans AS. 1976. Causation and disease: The Henle-Koch postulates revisited. *Yale Journal of Biology and Medicine* 49(2):175–195.

Gunderson CH, Lehmann CR, Sidell FR, Jabbari B. 1992. Nerve agents: A review. *Neurology* 42(5):946–950.

Hill AB. 1965. The environment and disease: Association or causation? *Proceedings of the Royal Society of Medicine* 58:295–300.

IARC (International Agency for Research on Cancer). 2004. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Tobacco Smoke and Involuntary Smoking. Volume 83.* Lyon, France: IARC.

IOM (Institute of Medicine). 1991. *Adverse Effects of Pertussis and Rubella Vaccine.* Washington, DC: National Academy Press.

IOM. 1994a. *DPT Vaccine and Chronic Nervous System Dysfunction: A New Analysis.* Washington, DC: National Academy Press.

IOM. 1994b. *Veterans and Agent Orange: Health Effects of Herbicides Used in Vietnam.* Washington, DC: National Academy Press.

IOM. 1996. *Veterans and Agent Orange: Update 1996.* Washington, DC: National Academy Press.

IOM. 1999. *Veterans and Agent Orange: Update 1998.* Washington, DC: National Academy Press.

IOM. 2000a. *Gulf War and Health, Volume 1: Depleted Uranium, Pyridostigmine Bromide, Sarin, and Vaccines.* Washington, DC: National Academy Press.

IOM. 2000b. *Clearing the Air: Asthma and Indoor Air Exposures.* Washington, DC: National Academy Press.

IOM. 2001. *Veterans and Agent Orange: Update 2000.* Washington, DC: National Academy Press.

IOM. 2003a. *Gulf War and Health, Volume 2: Insecticides and Solvents.* Washington, DC: The National Academies Press.

IOM. 2003b. *Veterans and Agent Orange: Update 2002.* Washington, DC: The National Academies Press.

PAC (Presidential Advisory Committee on Gulf War Veterans' Illnesses). 1996. *Presidential Advisory Committee on Gulf War Veterans' Illnesses: Final Report.* Washington, DC: US Government Printing Office.

Rostker B. 2000. US demolition of operations at Khamisiyah. Washington, DC: Department of Defense, December 7. http://www.gulflink.osd.mil/khamisiyah/index.html.

Somani SM. 1992. *Chemical Warfare Agents*. New York: Academic Press.

Susser M. 1973. *Causal Thinking in the Health Sciences: Concepts and Strategies of Epidemiology*. New York: Oxford University Press.

Susser M. 1977. Judgement and causal inference: Criteria in epidemiologic studies. *American Journal of Epidemiology* 105(1):1–15.

Susser M. 1988. Falsification, verification, and causal inference in epidemiology: Reconsideration in the light of Sir Karl Popper's philosophy. In: Rothman KJ, ed. *Causal Inference*. Chestnut Hill, MA: Epidemiology Resources. Pp. 33–58.

Susser M. 1991. What is a cause and how do we know one? A grammar for pragmatic epidemiology. *American Journal of Epidemiology* 133(7):635–648.

Wegman DH, Woods NF, Bailar JC. 1997. Invited commentary: How would we know a Gulf War syndrome if we saw one? *American Journal of Epidemiology* 146(9):704–711.

Winkenwerder W. 2002. US demolition operations at Khamisiyah. Final Report. Washington, DC: Department of Defense. http://www.gulflink.osd.mil/khamisiyah/index.html.

2

Toxicology

The basic toxicology of sarin and cyclosarin is discussed in *Gulf War and Health: Volume 1*, hereafter referred to as *GW1* (IOM, 2000). That background information is reviewed briefly here, and the review is followed by a discussion of data published since the preparation of *GW1*, focusing on data that might be relevant to low-dose exposures to sarin. Because sarin and cyclosarin have the same mechanism of action and toxic effects, differing mainly in potency, data on the two compounds are discussed together.

PHYSICAL AND CHEMICAL PROPERTIES

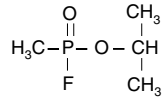
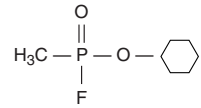
As discussed in *GW1* (IOM, 2000), sarin (GB; *o*-isopropyl methylphosphonofluoridate) and cyclosarin (GF; cyclohexyl methylphosphonofluoridate) are potent neurotoxicant organophosphate esters. Their chemical structures and properties are presented in Table 2-1.

TOXICOKINETICS

Absorption and Metabolism

Organophosphorus (OP) compounds are absorbed rapidly and produce local and systemic effects. Exposure to sarin or cyclosarin can be fatal within minutes to hours. In vapor or liquid form, sarin can be, respectively, inhaled or absorbed through the skin, eyes, or mucous membranes (Stewart and Sullivan, 1992). Because of its extreme potency, sarin is lethal to 50% of exposed people at doses

TABLE 2-1 Physical and Chemical Properties of Sarin and Cyclosarin

Characteristic	Sarin	Cyclosarin
Chemical name	Isopropyl methylphosphonofluoridate	*O*-Cyclohexyl-methylfluorophosphonate
Synonyms	Methylphosphonofluoridate, isopropyl ester	Cyclohexyl methylphosphonofluoridate (CMPF)
Chemical formula	$C_4H_{10}FO_2P$	$C_7H_{14}FO_2P$
Chemical structure		
Molecular weight	140.10	180.2
CAS Registry Number	107-44-8	329-99-7
Physical state	Colorless liquid	Liquid
Solubility in water, g/L	Miscible with water	0.37% (20°C); almost entirely insoluble in water
Vapor pressure	2.10 mm Hg at 20°C	0.044 mm Hg at 25°C

Data from DA, 1990. Table modified from NRC, 2003.

of 100–500 mg through the skin, or 50–100 mg-min/m^3 by inhalation (in a person who weighs about 70 kg) (Somani, 1992).

In the blood, sarin interacts with several esterases. Some, such as paraoxonase, hydrolyze sarin to inactive metabolites (Davies et al., 1996; Lotti, 2000). Two others—acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE)—irreversibly bind to sarin. Those esterases in the blood are often described as false targets—by binding irreversibly to sarin, AChE and BuChE sequester sarin in the blood, thereby preventing some or all of it from reaching the central nervous system (CNS), depending on the dose (Spencer et al., 2000).

Distribution and Elimination

Animal data obtained by using radioactively labeled sarin indicate that sarin rapidly (within 1 min) distributes to the brain, lungs, heart, diaphragm, kidneys, liver, and plasma; the greatest concentrations are found in the last three (Little et al., 1986). The concentrations in all tissues decline rapidly; a decrease of 85% within 15 min was followed by a second, more gradual decline. The initial, rapid decline appears to be mediated by metabolism, not urinary elimination of the parent compound, inasmuch as about half the labeled sarin was associated within

the first minute with the major sarin metabolite isopropyl methylphosphonic acid (IMPA). A recent study in guinea pigs indicated that sarin stereoisomers reach the blood rapidly after nose-only exposures and then gradually decline (Spruit et al., 2000). The kidneys are the major route of elimination of sarin and its metabolites (Little et al., 1986). Urinary elimination of sarin is rapid (terminal elimination half-life, 3.7 ± 0.1 h); almost all the administered dose of sarin was retrieved from the urine in metabolite form after 2 days (Shih et al., 1994).

Minami et al. (1997) detected IMPA in urine of humans after a terrorist attack on the Tokyo subway system with sarin; peak concentrations were measured 10–18 h after exposure. Evidence of distribution of sarin to the human brain was found in 4 of the 12 people who died after exposure (Matsuda et al., 1998).

BIOMARKERS OF EXPOSURE

Inhibition of blood cholinesterases can be used as a biomarker of exposure to sarin. Although high doses of sarin inhibit both AChE and BuChE, at lower doses sarin preferentially inhibits AChE, making AChE inhibition a more sensitive biomarker of sarin exposure than BuChE inhibition (Sidell and Borak, 1992). Because inhibition of blood cholinesterases is a common feature of sarin, other OP compounds, and some other compounds, cholinesterase inhibition is not a specific biomarker of sarin exposure. Blood esterase activity returns to normal 1–3 months after exposure and this limits its utility as a biomarker to a short time after exposure (Grob, 1963). Fidder et al. (2002) developed a more specific biomarker of sarin poisoning by measuring organophosphate-inhibited BuChE in blood.

Sensitive methods for detecting methylphosphonic acids, which are metabolites of sarin, in blood or urine for use as a biomarker of sarin exposure have been developed (Shih et al., 1991; Black et al., 1994; Fredriksson et al., 1995; Tørnes et al., 1996; Black and Read, 1997, 1998) and used by Japanese researchers in the aftermath of the Tokyo terrorism incident (Minami et al., 1997, 1998; Noort et al., 1998). Those methods have the advantage of having more specificity than use of cholinesterase inhibition, however, they are limited by the fact that methylphosphonic acids are eliminated from the body within several days after exposure to sarin.

Researchers have also measured the amount of phosphyl moiety released upon reactivation of the phosphylated BuChE or AChE by fluoride ion or other treatments (Nagao et al., 1997; Polhuijs et al., 1997; Matsuda et al., 1998). Measurement of the phosphyl moiety allows the type and amount of the OP compound exposure to be determined, and can be used longer after a poisoning episode than by detection of sarin metabolites. More recently, Fidder et al. (2002) measured phosphylated nonapeptides created following pepsin digestion of inhibited BuChE.

MECHANISMS OF TOXICITY

Inhibition of Acetylcholinesterase

The principal mechanism of acute toxicity of sarin and cyclosarin, as of other OP compounds, is inhibition of AChE. AChE is responsible for the hydrolysis of acetylcholine (ACh) at the synapse, and inhibition of AChE leads to a rise in ACh and overstimulation at cholinergic synapses (Somani, 1992; Lotti, 2000; Spencer et al., 2000). At those synapses, the ACh binds and activates muscarinic and nicotinic receptors, the two major subtypes of ACh receptors. Sarin inhibits AChE by phosphorylating a serine hydroxyl on the ester portion of the active site of the enzyme. The phosphorylated enzyme is hydrolyzed very slowly, with a half-life of reactivation of hours to days (Gray, 1984). The phosphorylated enzyme can undergo a second process, called aging, by loss of an alkyl group (dealkylation). Aging occurs within about 5 h of sarin exposure (Sidell and Borak, 1992). After aging has occurred, the phosphorylated enzyme is resistant to cleavage or hydrolysis and can be considered irreversibly inhibited. Recovery of AChE function occurs only with synthesis of new enzyme. Most of the effects of sarin, including the acute cholinergic syndrome, are thought to be mediated by the excess ACh at the synapse.

Other Mechanisms

For decades, as discussed in *GW1* (IOM, 2000), researchers observed puzzling relationships between the extent of neurobehavioral toxicity and the degree of inhibition of AChE. For example, sarin-induced tremor has a slight correlation with AChE inhibition in rat striatum, but chewing, hind-limb abduction, and convulsions have no clear correlation (Hoskins et al., 1986). Some sarin-treated rats with 90% inhibition of AChE in the striatum of the brain had no convulsions or hind-limb abduction, but rats with less enzyme inhibition exhibited both. On the basis of those findings, researchers have concluded that mechanisms other than the inhibition of AChE might also contribute to toxicity induced by sarin and other organophosphates. The difficulty, however, has been in differentiating among effects mediated directly by sarin and effects that are secondary to its inhibition of AChE.

Electrophysiologic experiments have indicated that sarin (in picomolar concentrations) can interact with one subtype of ACh receptors, the muscarinic ACh receptors (Rocha et al., 1998; Chebabo et al., 1999). That interaction appears to be direct and is not associated with the inhibition of AChE.

Several studies suggest that sarin can alter the concentrations of neurotransmitters other than ACh. In most of them, however, the neurotransmitter effects are seen in brain regions where there are cholinergic synapses and could be secondary to AChE inhibition (Dasheiff et al., 1977; Fernando et al., 1984;

Somani, 1992). Sarin-like agents have also been shown to alter second-messenger systems in rat brains, including activating phospholipase C gamma (Niijima et al., 1999), mitogen-activated protein kinase (MAPK), and c-Jun N-terminal kinase (JNK) protein activity (Niijima et al., 2000). The mechanisms underlying those effects are unknown. A recent study in rats (Abu-Qare and Abou-Donia, 2001) showed that concurrent exposure to sarin and pyridostigmine bromide produced biomarkers of oxidative stress (3-nitrotyrosine and 8-hydroxy-2′-deoxyguanosine in rats), raising the possibility that some effects in rats could be mediated by oxidative stress.

ACUTE HUMAN EXPOSURES TO ORGANOPHOSPHORUS COMPOUNDS

Immediate Effects

As discussed in *GW2*, clinical signs of toxicity associated with organophosphate-induced inhibition of AChE depend on dosage. Toxicity in humans and animals includes the signs associated with overstimulation of muscarinic receptors of the autonomic nervous system by ACh: SLUD (salivation and sweating, lacrimation, urination, and defecation), emesis, and bradycardia. AChE inhibition can also cause overstimulation (which can be followed by depression) of nicotinic receptors at neuromuscular junctions and autonomic ganglia and result in ataxia and fasciculations that, at higher dosages, can be followed by flaccid paralysis. Electromyographic changes can be observed after acute poisoning because nicotinic sites in muscles are affected; the changes include decreases in amplitude and increases in peak latencies in nerve conduction (Baker and Wilkinson, 1990; Gallo and Lawryk, 1991; Kaloianova and El Batawi, 1991). Stimulation of autonomic ganglia can also cause hypertension. As is the case at neuromuscular junctions, excess ACh in the CNS causes stimulation that can be followed by depression. Overstimulation can be manifested as nervousness, delirium, hallucinations, and psychoses. Obvious signs do not generally appear until nervous system AChE inhibition approaches 70%.

Not all exposed people show all signs, and signs can vary with the OP compound, dose, route of exposure, and species. Signs often appear within minutes or hours, but they might not appear for several days. Signs can last for minutes to weeks and can be followed by full recovery from obvious manifestations of cholinergic poisoning. If death occurs, it is due to respiratory failure, usually as a result of a combination of the autonomic effects mediated by the muscarinic and nicotinic ACh receptors and the effects of ACh at CNS receptors. Those effects can include excessive fluid in the respiratory tract, paralysis of the respiratory muscles, and depression of the respiratory centers of the CNS.

Delayed Effects

Intermediate Syndrome

Clinical manifestations of acute AChE inhibition in humans or animals are not generally long-lasting or delayed, but there are exceptions. An "intermediate syndrome" has been described after severe poisoning: muscle weakness that occurs about 16–120 h after exposure and 7–75 h after the onset of acute poisoning symptoms (Shailesh et al., 1994; He et al., 1998). Overstimulation of nicotinic receptors and then depression at neuromuscular junctions and muscle necrosis might be contributing factors. The muscle weakness can become severe and result in respiratory insufficiency. Recovery occurs, if respiration can be sustained, but it can take weeks. The intermediate syndrome has been reported in humans after exposure to malathion and diazinon (Gallo and Lawryk, 1991).

Organophosphorus-Induced Delayed Neuropathy

Another type of toxicity caused by a few OP compounds is a progressive, irreversible delayed neuropathy termed organophosphate-induced delayed neuropathy (OPIDN). OPIDN can occur in many species, including humans. Clinical manifestations of OPIDN include progressive ataxia that develops weeks to months after exposure. Lesions are found in peripheral nerves and the spinal cord (Ehrich and Jortner, 2001). OPIDN becomes manifest about 1–4 weeks after an acute exposure to some organophosphates; motor symptoms of ataxia and flaccid paralysis of the lower extremities are exhibited. Symptoms persist for up to a year and may be permanent in severe cases (De Bleecker et al., 1992).

OPIDN is thought to be mediated by effects on an enzyme known as neuropathy target esterase (NTE) (Somani, 1992; Moore, 1998; Lotti, 2000). OPIDN occurs only if OP compounds inhibit NTE sufficiently, and essentially irreversibly, within hours of exposure. Inhibition of NTE is not related to inhibition of AChE. OP compounds are tested for their potential to cause OPIDN before they are registered for use as insecticides, so most commercially available insecticides do not inhibit NTE.

Other Delayed Effects

As discussed in *GW2*, some studies have reported other persistent symptoms after poisoning with OP compounds or symptoms that appear 5–10 years after a poisoning episode, including neurologic and visual deficits, behavioral alterations, and impairment of cognition. Those effects, however, might be confounded by other factors or result from inappropriate study designs (see Baker and Wilkinson, 1990; Gallo and Lawryk, 1991; Kaloianova and El Batawi, 1991; Chambers and Levi, 1992; Ecobichon and Joy, 1994; Abou-Donia, 1995; Eyer, 1995; Jamal, 1997; Lotti, 2001 for reviews). Although some latent effects have

been noted in laboratory rats, the symptoms reported in people have been difficult to verify in animal studies partly because of difficulties in replication of exposures and extrapolation of end points from humans to animals (see Ballantyne and Marrs, 1992; Bushnell et al., 1993; Ecobichon and Joy, 1994; Gallo and Lawryk, 1991; Marrs et al., 1996; Mattsson et al., 1996; Maurissen et al., 2000 for reviews).

EXPERIMENTAL STUDIES

Most animal studies of sarin and cyclosarin examine the effects at lethal, near-lethal, or maximum tolerated doses (MTDs).[1] Those high doses produce the acute cholinergic syndrome, in many cases necessitate pharmacologic intervention to prevent death, and are not useful in distinguishing between primary damage caused by the compound and secondary damage caused by hypoxic events after convulsions. There is no evidence that any Gulf War soldiers had the acute cholinergic syndrome, so studies of acute, high-dose exposure to sarin or cyclosarin are only briefly mentioned, and this section focuses more on studies—published since the preparation of *GW2*—of the long-term effects of low-dose exposures to compounds that are more relevant to the situation in the Gulf War. This section is organized by the end point studied, and also by studies that look at short-term effects and those that examine effects that persist for weeks or months after a single or short-term exposure. The studies investigating persistent effects are more relevant to the veterans' situation.

Lethality Studies

In animals, sarin and cyclosarin in microgram quantities are acutely toxic and fatal in a matter of minutes. There is some variability, depending on the species and the route of administration. Table 2-2 outlines some of the doses and routes of administration that produce acute lethality (within 24 h) in animal species tested. The LD_{50} of cyclosarin in mice (243 μg/kg) is somewhat higher than that of sarin (170 μg/kg) (Clement, 1992). The immediate cause of death from sarin poisoning is respiratory arrest (Rickett et al., 1986); a study by Duncan et al. (2001) indicates that in swine it results from central respiratory failure.

Neurotoxicity

Short-Term Neurotoxicity

Sarin's short-term behavioral effects are dose-dependent. Sarin has led to conditioned flavor aversion (at doses greater than 70 μg/kg) and to decreased

[1]The MTD is the highest dose used during a long-term study that will not alter the life span of the animal and suppresses body weight gain only slightly (10%) in a 90-day subchronic study.

TABLE 2-2 Acute Lethality of Sarin Administered to Various Species

Species	Route[a]	LD_{50}, μg/kg	Reference
Rat	s.c.	158–165	Landauer and Romano, 1984; Singer et al., 1987; Somani, 1992
Mouse	s.c.	160–170	Clement, 1991
Mouse	i.m.	179	Somani, 1992
Mouse	i.v.	109	Little et al., 1986; Tripathi and Dewey, 1989
Mouse	Inhalation	600 mg/min per m^3	Husain et al., 1993
Guinea pig	s.c.	53 (divided doses)	Fonnum and Sterri, 1981; Somani, 1992
Hen	Oral	561	Bucci et al., 1993
Hen	s.c.	16.5–16.7	Gordon et al., 1983
Cat	s.c.	30–35	Goldstein et al., 1987

[a]i.m. = intramuscular; i.v. = intravenous; s.c. = subcutaneous.

motor coordination in rats as measured by rotarod performance (at 98 μg/kg; Landauer and Romano, 1984). It has led to increased spontaneous locomotion at 61 μg/kg but decreased locomotor activity at higher doses immediately after treatment (Landauer and Romano, 1984); Nieminen et al. (1990) found 50 μg/kg, but not 12.5 μg/kg, to decrease locomotion until 6 h after intraperitoneal administration, and to decrease some behaviors 40–50 min after injection.

As discussed in *GW1*, short-term behavioral effects have been examined in the marmoset, a nonhuman primate. Doses at 33–55% of the LD_{50} disrupted the performance of animals' food-reinforced visually guided reaching response. Performance returned to normal by 24 h after sarin administration (D'Mello and Duffy, 1985).

The only other studies of short-term behavioral consequences of low-dose exposures in nonhuman primates were carried out with soman, an OP nerve agent that also inhibits AChE. Hartgraves and Murphy (1992) studied the effects of different dosing regimens—which did not produce signs of acute toxicity—on equilibrium performance as measured on the primate equilibrium platform (PEP). This device requires the primate to manipulate a joystick to keep a rotating platform as level as possible. Doses below 2.0 μg/kg did not induce and doses above 2.75 μg/kg did induce decrements in PEP performance. Decrements were measured for 5 days after soman administration, but performance later returned to normal. Those findings, although not from sarin, are reported here because vestibular dysfunction has been reported as a long-term effect in humans after sarin exposure.

Since the preparation of *GW1*, Hulet et al. (2002) tested a functional observational battery and reported EEG results in guinea pigs after a single injection of sarin (0.3, 0.4, 0.5, or 0.6 times the LD_{50}). Few changes were seen in the battery, but at $0.4LD_{50}$ and above, responses to an approaching pencil and to a rump-touch were different from controls, and they did not adjust to handling. No changes were seen up to $0.5LD_{50}$, but $0.6LD_{50}$ led to EEG evidence of seizures. Symptoms of the acute cholinergic syndrome were evident at $0.5LD_{50}$ and above.

Studies have looked at the effects of a single dose of sarin on glial markers. Damodaran et al. (2002) studied the effects of sarin (intramuscular injection at 50 μg/kg per milliliter vehicle) on mRNA expression of astroglial markers 1 and 2 h and 1, 3, and 7 days after treatment. Glial fibrillary acidic protein and vimentin were increased in the areas of the brain studied (cortex, midbrain, cerebellum, brainstem, and spinal cord); vimentin induction occurred sooner. Some effects on expression of both could still be detected 7 days after treatment.

Those data indicate that sarin exposure in animals can have effects on neurobehavioral and neurotoxic endpoints. No clear pattern of effects, however, emerges from those studies and their relevance to humans is unknown.

Persistent Neurotoxicity

As discussed in *GW1* (IOM, 2000), long-term changes in the electroencephalogram (EEG) of rhesus monkeys have been seen after a single high dose of sarin (5 μg/kg) or a series of 10 small doses (1 μg/kg per week) (Burchfiel et al., 1976; Burchfiel and Duffy, 1982). The high dose was sufficient to produce the acute cholinergic syndrome, whereas each small dose produced few, if any, signs of acute poisoning. Changes persisted for a year after sarin administration, although they did not appear to have any behavioral or psychologic significance. In a later study in marmosets, no statistically significant changes in EEG were detected, but the increase in the beta 2 amplitude (22–40 Hz) approached statistical significance ($p = 0.07$) (Pearce et al., 1999). The dose did not produce a decrement in touchscreen-mediated discrimination tasks, which are indicators of cognitive functioning.

Since the preparation of *GW1*, research has been conducted in animals with sarin exposures designed to resemble those which might have occurred in the Gulf War. The studies were specifically designed to investigate possible effects of low-level exposure to sarin that persist for weeks or months after the exposure ends. Henderson et al. (2001; extended abstract encompassing other studies) studied locomotor activity and body temperature in rats exposed only intranasally to sarin (0.2 or 0.4 mg/m^3 of air for 1 h/day for 1, 5, or 10 days) in the presence or absence of heat stress (32° C). The higher concentration (0.4 mg/m^3) is one-tenth the lethal concentration (LCt_{50}).[2] The animals were monitored continually

[2]The concentration that is lethal to 50% of the animals.

for a month after exposure, and the data were grouped. No consistent effects were seen. Using the same treatment protocol, Henderson et al. (2002) looked at brain histopathologic effects in rats 30 days after exposure. Heat stress, but not sarin treatment, decreased weight gain and pulmonary function. No lesions or evidence of apoptosis were present. No effect was seen on total brain AChE measurements (from homogenates), but region-specific staining for AChE was decreased in the cerebral cortex, striatum, olfactory bulb, and CA1 region of the hippocampus. Thus, in general, the forebrain concentrations of AChE were most affected. Brain cytokine concentrations (interleukin (IL)-1β, IL-6, and tumor necrosis factor-α) were affected by both sarin treatment and heat stress; this is consistent with evidence of immunosuppression seen in other experiments (decreased anti-sheep RBC antibody forming cell response and suppression of T cell response) (Kalra et al., 2002).

Receptor density was measured for the M1, M2, and M3 subtypes of muscarinic receptors (Henderson et al., 2002). M1 receptors were decreased in a dose-dependent manner. No changes were seen on day 1 after 5 days of treatment, but a decrease in M1 receptors was seen in the olfactory tubercle 30 days after the highest dose. With heat stress, there were also dose-dependent decreases in M1 receptor density in the frontal cerebral cortex, olfactory tubercle, anterior olfactory nucleus, striatum, dentate gyrus, and CA1 region of the hippocampus 30 days after treatment. No changes were seen in M2 receptor densities with any treatments. Sarin did not affect M3 receptor density under normal conditions, but under heat stress there was an increase in the number of M3 receptors at day 1 and day 30 after 5 days of treatment in the frontal cortex, olfactory tubercle, and anterior olfactory nucleus and throughout the striatum. At 30 days, there was also an increase in M3 receptor density in the CA1 region of the hippocampus. Those studies provide the results most relevant to potential effects of exposures in the Gulf War. Although the results on receptor density are not such that they alter the conclusions of this committee regarding the strength of the association between exposure to sarin and neurologic health outcomes, they are suggestive of a potential mechanism through which sarin could cause long-term effects on the nervous system and indicate the desirability of future toxicologic and epidemiologic research. Further studies of concomitant exposure to stressors (e.g., heat) or other chemicals and sarin should also be conducted.

In addition to the studies by Henderson and colleagues, behavioral effects of single and repeated (three times in 1 week) doses of sarin (0, 0.8, 1.35, or 2.5 μg/L) have been investigated in an inhalation chamber since the preparation of *GW1*. The performance of rats in a T-maze was somewhat affected after exposure to sarin (Kassa et al., 2001a) or sarin plus oximes (Krejcova et al., 2002), as was performance of rats in a Y-maze after exposure to sarin (Kassa et al., 2001b) or to sarin plus oximes (Kassa et al., 2002). The performance of some of the controls, however, was also lower than expected in some of those studies with oximes, and many of the effects seen were reversed by 3 months.

In summary, some changes in EEG and histopathology persisted months to a year after exposure in animals. Those effects, however, did not appear to be associated with detectable behavioral changes or clinically-relevant effects.

Delayed Neurotoxicity

As discussed previously, exposure to some organophosphates produces a delayed neurotoxic syndrome known as organophosphate-induced delayed neuropathy. In some animal models, massive doses of sarin can cause delayed neurotoxicity, which is manifested in ataxia and paralysis days to weeks after a single high exposure or multiple lower exposures (Somani, 1992; Lotti, 2000; Spencer et al., 2000). The doses of most OP compounds capable of producing those neurotoxic effects in experimental animals are typically higher than the lethal dose. Therefore, to study delayed neurotoxicity, most species must be protected from death through pharmacologic and other interventions.

Table 2.3 summarizes findings of animal studies of OPIDN or other forms of delayed neurotoxicity after administration of sarin reviewed in *GW1* (IOM, 2000). Sarin produced delayed neurotoxicity in six studies. In four of them, the doses were either the lethal dose or at least 30 times the lethal dose (Davies et al., 1960; Davies and Holland, 1972; Gordon et al., 1983; Willems et al., 1983). Animals displayed severe signs of acute cholinergic toxicity but were protected from death by administration of atropine and other agents. In two studies, however, sublethal doses were administered. Researchers administered sarin to mice (Husain et al., 1993) and white leghorn hens (Husain et al., 1995) for 10 days. At no time did sarin-exposed mice show signs of cholinergic toxicity, although AChE activity was inhibited by 27% (blood) and 19% (brain). No indication was provided on whether cholinergic symptoms were observed in the hens, but platelet AChE activity was inhibited by 72%. Animals developed muscular weakness of the limbs and slight ataxia within 14 days of the beginning of the study. NTE was inhibited in the brain, spinal cord, and platelets, and the spinal cord exhibited axonal degeneration. In several studies, however, sarin did not produce delayed neurotoxicity. Crowell et al. (1989) attributed the negative findings in hens to sarin's inability to inhibit brain NTE substantially at nonlethal doses.

Taken together, the findings indicate that sarin can cause OPIDN in some animal species, particularly at doses that produce otherwise lethal effects.

Immunotoxicity

Kalra et al. (2002) studied T-cell responses to sarin in Fischer 344 rats. Nose-only exposure of rats to sarin (0, 0.2, or 0.4 mg/m^3; 1 h/day for 1, 5, or 10 days) had some effects on T cells isolated from spleen of the rats 1 day after the final sarin treatment. Sarin at 0.2 or 0.4 mg/m^3 for 5 or 10 days decreased antibody-forming cells and T cell proliferation, but the number and distribution of cells

were unchanged. Intracellular calcium responses and T cell proliferation were also affected by some treatments. Sarin decreased corticosterone (CORT) concentrations; this indicates that the effect was not mediated by the hypothalamic-pituitary-adrenal axis, and experiments with a ganglionic blocker suggested an autonomic effect.

Recent studies have investigated more long-term effects of sarin on the immune system. Kassa et al. (2000, 2001c) demonstrated modest and inconsistent effects on lymphocyte proliferation and production of *N*-oxides in rats 3 months after a single or repeated (three times in one week) 1-h inhalation-chamber exposure (0.8, 1.25, or 2.5 μg/L).

The effects of sarin on the immune system of animals, therefore, are inconsistent.

Genotoxicity

A study of the genotoxicity of sarin showed no evidence of genotoxicity (mutagenesis, chromosomal damage, unscheduled DNA synthesis, or sister chromatid exchange) (Goldman et al., 1988). In one study in rats, DNA synthesis was not changed, but an increase in unscheduled DNA repair was observed, although problems with controls and variability provide less confidence in those results (Klein et al., 1987). No studies on the genotoxicity of sarin have been published since *GW1* (IOM, 2000).

Cancer

As discussed in *GW1* (IOM, 2000) a standard subchronic (90-day) toxicology study of sarin was performed at the National Center for Toxicological Research (Bucci and Parker, 1992; Bucci et al., 1992). This subchronic study is discussed here because of some endpoints seen, but such a study is not adequate for determining the carcinogenicity of a chemical. A lack of tumours in such a study cannot be interpreted to indicate that the chemical is not a carcinogen. Rats were administered sarin in two formulations (type I stabilized with tributylamine and type II with diisopropylcarbodiimide) at three doses: the MTD, MTD/2, and MTD/4—corresponding to 300, 150, and 75 μg/kg per day, respectively—given by gavage. Both formulations produced profound inhibition of AChE and some deaths. No neoplastic lesions were detected after type I sarin, but nonneoplastic lesions (necrosis in the cerebrum related to hypoxia) were detected and were thought to be the cause of death in 3 of 36 female rats (1 at 75 μg/kg, and 2 at 300 μg/kg.). Type II sarin was associated with one neoplastic lesion, a lymphoma, in a male in the high-dose group.

No chronic animal studies have been conducted to determine the carcinogenic effects of exposure to sarin.

TABLE 2-3 Delayed Neurotoxicity of Sarin

Species	Dose, μg/kg	Route of Administration[a]	Frequency and/or Duration
Chicken	25 (LD_{50})	i.m.	1×/day for 26–28 days
Hen	500–2,500	i.m. (20% of total dose given)	1×/day for 5 days
Hen	252	s.c.	1×
	504–1,962	s.c.	1×
Chicken	70.2–281	Gavage	1×
	23–94	Gavage	1×/week for 3 weeks
Hen	50 (LD_{50}/10)	s.c.	1×/day for 10 days
Hen	600	i.m.	1×/day for 2 days
	900		1×/day for 3 days
	1,500		1×/day for 5 days
	900		1×/day for 1 day
	1,200		1×/day for 1 day
Rat	75–300	Gavage	5×/week for 13 weeks
Mouse	5 mg/m^3	Inhalation	20 min for 10 days
Cat	1,000	s.c.	1×
	3.5	s.c.	1×/day for 10 days
	7	s.c.	1×/day for 5 days

[a]i.m. = intramuscular; i.p. = intraperitoneal; i.v. = intravenous; s.c. = subcutaneous.
[b]NA = not available; PAD = dodecyl iodide salt of P2S; P2S = pralidoxime mesylate, 2-hydroxyiminomethyl-N-methylpyri-dinium methyl methanesulfonate.
[c]DN = delayed neuropathy.
[d]No hens were ataxic at 500 μg/kg. Figures not provided for doses higher than 1,000 μg/kg.

Reproductive or Developmental Toxicity

As discussed in *GW1*, sarin appears to have no reproductive effects in rats, rabbits, or dogs (LaBorde et al., 1996; Jacobson et al., 1959).

GENETIC SUSCEPTIBILITY

One of the mechanisms of sarin inactivation is hydrolysis by the enzyme paraoxonase (PON1), an esterase synthesized and secreted by the liver. It is

Protection[b]	Neurobehavioral Outcomes[c]	Reference
Atropine, P2S, PAD	5/8 slight ataxia	Davies and Holland, 1972
Atropine, P2S dose of 1,000 μg/kg[d]	9/28 ataxia at minimal	Davies et al., 1960
Physostigmine, atropine, P2S	0/4 ataxia 12/12 ataxia to paralysis	Gordon et al., 1983
Atropine Atropine	None None	Bucci et al., 1993
None	Moderate ataxia[e]	Husain et al., 1995
Atropine, Physostigmine, P2S	0/4 DN 1/3 DN 8/9 DN 3/4 DN 4/4 DN	Willems et al., 1983
NA	None	Bucci and Parker, 1992; Bucci et al., 1992
None	Slight ataxia[f]	Husain et al., 1993
Physostigmine, atropine None None	None None[g] None[g]	Goldstein et al., 1987

[e]Study does not report how many of five dosed animals developed moderate ataxia.

[f]Study does not report how many of six dosed animals developed slight ataxia.

[g]No behavioral signs of neurotoxicity, but sarin decreased conduction velocity of muscle spindle afferents and altered frequency response of primary and secondary nerve endings.

found in humans in the brain and the blood. The human PON1 gene has polymorphisms at positions 192 (*Arg/Gln*) and 55 (*Leu/Met*) that affect serum PON1 activity (Furlong et al., 1993). Because human serum PON1 catalyzes the hydrolysis of OP insecticides and nerve gases such as sarin, those polymorphisms might substantially alter a person's susceptibility to the toxicity of the chemicals. The polymorphism at position 192 accounts for three genotypes (QQ, RR, and QR) related to the catalytic properties of two forms of the PON1 enzyme (types R and Q allozymes), which hydrolyze some organophosphates at different rates.

The R allozyme (Arg_{192}) hydrolyzes the organophosphate paraoxon at a high rate; however, it has a low activity against OP nerve agents such as sarin and soman (Davies et al., 1996). Lower activity means that more sarin would be bioavailable to exert its anticholinesterase effects. The Q allozyme has high activity against OP nerve agents and low activity against paraoxon. Thus, people with the Q allozyme (genotype QQ or QR) are expected to have greater hydrolysis of sarin than people homozygous for the R allele (genotype RR). Animal studies support the role of PON1 in protection against the toxicity of some OP compounds (Costa et al., 2003). The prevalence of the R allele is about 0.3 in Caucasian populations but 0.66 in the Japanese population (Yamasaki et al., 1997). Because that form is associated with low hydrolysis of sarin, the authors hypothesized that it could make the Japanese population more sensitive to the toxicity of sarin, which might contribute to their morbidity and mortality after the terrorist attacks in Japan. Yamada et al. (2001), however, reported that of 10 of the victims of the Tokyo attack, 7 expressed the PON1 Q allele (6 QR, 1 QQ). The genotype that confers high hydrolyzing activity toward sarin, therefore, did not appear to play a role in protecting those exposed against the toxicity of sarin.

The relationship between illness in Gulf War veterans and the PON1 genotype and serum AChE activity has been investigated by Haley et al. (1999). The enzyme activity, or ability to metabolize ACh, can be quantified in serum samples from the veterans. That activity is, in part, a function of the genotype of the veteran. Ill veterans (n = 25) were more likely than controls (n = 20) to possess the R allele (genotype RR or QR; OR, 3.50; CI, 0.26–2.80) and to exhibit lower PON1 type Q arylesterase activity. That study raises the possibility that the R allele represents a risk factor for illness in Gulf War veterans, but in a nested case–control study, Hotopf et al. (2003) did not find any differences in PON1 activity between symptomatic and asymptomatic Gulf War veterans. Those researchers studied symptomatic Gulf War veterans, healthy Gulf War veterans, symptomatic Bosnia peacekeeping veterans, and symptomatic nondeployed military controls. The main outcome measures were PON1 activity and genotype for PON1-55 and -192. The authors observed statistically significant differences in PON1 activity among the four groups, but the two gulf groups did not differ in PON1 activity. However, those deployed to the gulf had significantly lower PON1 activity than the non-Gulf War groups (median difference, 70.9; 95% CI, 20.2–121.5; p = 0.012); the differences were not explained by PON1 polymorphisms. PON1 activity was lower in Gulf War veterans than in military control groups. The effect is independent of ill health in Gulf War veterans.

Those studies do not entirely clarify the role of PON1 in Gulf War veterans. A study by Mackness et al. (2000) suggests that symptomatic Gulf War veterans have lower PON1 activity, but this is not explained by the various genotypes in Hotopf et al. (2003). Nonetheless, the decreased activity of PON1 would result in an increased susceptibility to OP insecticides and gases, such as sarin.

REFERENCES

Abou-Donia MB. 1995. Involvement of cytoskeletal proteins in the mechanisms of organophosphorus ester-induced delayed neurotoxicity. *Clinical and Experimental Pharmacology and Physiology* 22(5):358–359.

Abu-Qare AW, Abou-Donia MB. 2001. Combined exposure to sarin and pyridostigmine bromide increased levels of rat urinary 3-nitrotyrosine and 8-hydroxy-2'-deoxyguanosine, biomarkers of oxidative stress. *Toxicology Letters* 123(1):51–58.

Baker SR, Wilkinson CF. 1990. *The Effect of Pesticides on Human Health*. Princeton, NJ: Princeton Scientific Publishing Co., Inc.

Ballantyne B, Marrs TC. 1992. *Clinical and Experimental Toxicology of Organophosphates and Carbamates.* Oxford: Butterworth-Heinemann Ltd.

Black RM, Read RW. 1997. Application of liquid chromatography-atmospheric pressure chemical ionisation mass spectrometry, and tandem mass spectrometry, to the analysis and identification of degradation products of chemical warfare agents. *Journal of Chromatography A* 759:79–92.

Black RM, Read RW. 1998. Analysis of degradation products of organophosphorus chemical warfare agents and related compounds by liquid chromatography-mass spectrometry using electrospray and atmospheric pressure chemical ionisation. *Journal of Chromatography A* 794:233–244.

Black RM, Clarke RJ, Read RW, Reid MT. 1994. Application of gas chromatography-mass spectrometry and gas chromatography-tandem mass spectrometry to the analysis of chemical warfare samples, found to contain residues of the nerve agent sarin, sulphur mustard and their degradation products. *Journal of Chromatography A* 662(2):301–321.

Bucci TJ, Parker RM. 1992. *Toxicity Studies on Agents GB and GD (Phase 2): 90-Day Subchronic Study of GB (Sarin, Type II) in CD-Rats*. Available from the National Technical Information Service. NTIS/AD-A248 618/1.

Bucci TJ, Parker RM, Crowell JA, Thurman JD, Gosnell PA. 1992. *Toxicity Studies on Agents GB and GD (Phase 2): 90-Day Subchronic Study of GB (Sarin, Type I) in CD-Rats.* Available from the National Technical Information Service. NTIS/AD-A248 617/3.

Bucci TJ, Parker RM, Gosnell PA. 1993. *Toxicity Studies on Agents GB and GD (Phase 2): Delayed Neuropathy Study of Sarin, Type II, in SPF White Leghorn Chickens*. Available from the National Technical Information Service. NTIS/AD-A257357.

Burchfiel JL, Duffy FH. 1982. Organophosphate neurotoxicity: Chronic effects of sarin on the electroencephalogram of monkey and man. *Neurobehavioral Toxicology and Teratology* 4(6):767–778.

Burchfiel JL, Duffy FH, Van Sim M. 1976. Persistent effects of sarin and dieldrin upon the primate electroencephalogram. *Toxicology and Applied Pharmacology* 35(2):365–379.

Bushnell PJ, Pope CN, Padilla S. 1993. Behavioral and neurochemical effects of acute chlorpyrifos in rats: Tolerance to prolonged inhibition of cholinesterase. *Journal of Pharmacology and Experimental Therapeutics* 266(2):1007–1017.

Chambers JE, Levi PE, eds. 1992. *Organophosphates, Chemistry, Fate, and Effects*. San Diego: Academic Press.

Chebabo SR, Santos MD, Albuquerque EX. 1999. The organophosphate sarin, at low concentrations, inhibits the evoked release of GABA in rat hippocampal slices. *Neurotoxicology* 20(6):871–882.

Clement JG. 1991. Variability of sarin-induced hypothermia in mice: Investigation into incidence and mechanism. *Biochemical Pharmocology* 42(6):1316–1318.

Clement JG. 1992. Efficacy of various oximes against GF (cyclohexyl methylphosphonofluoridate) poisoning in mice. *Archives of Toxicology* 66(2):143–144.

Costa LG, Cole TB, Furlong CE. 2003. Polymorphisms of paraoxonase (PON1) and their significance in clinical toxicology of organophosphates. *Journal of Toxicol*ogy 41:37–45.

Crowell JA, Parker RM, Bucci TJ, Dacre JC. 1989. Neuropathy target esterase in hens after sarin and soman. *Journal of Biochemical Toxicology* 4(1):15–20.

DA (Department of the Army). 1990. *Occupational health guidelines for evaluation and control of occupational exposure to nerve agents GA, GB, GD and VX.* DA Pam 40-8. US Department of the Army, Headquarters. Washington, DC.

Damodaran TV, Bilska MA, Rahman AA, Abou-Doni MB. 2002. Sarin causes early differential alteration and persistent overexpression in mRNAs coding for glial fibrillary acidic protein (GFAP) and vimentin genes in the central nervous system of rats. *Neurochemical Research* 27(5):407–415.

Dasheiff RM, Einberg E, Grenell RG. 1977. Sarin and adrenergic–cholinergic interaction in rat brain. *Experimental Neurology* 57(2):549–560.

Davies DR, Holland P. 1972. Effect of oximes and atropine upon the development of delayed neurotoxic signs in chickens following poisoning by DFP and sarin. *Biochemical Pharmacology* 21(23):3145–3151.

Davies DR, Holland P, Rumens MJ. 1960. The relationship between the chemical structure and neurotoxicity of alkyl organophosphorus compounds. *British Journal of Pharmacology* 15:271–278.

Davies HG, Richter RJ, Keifer M, Broomfield CA, Sowalla J, Furlong CE. 1996. The effect of the human serum paraoxonase polymorphism is reversed with diazoxon, soman and sarin. *Nature Genetics* 14(3):334–336.

De Bleecker JL, De Reuck JL, Willems JL. 1992. Neurological aspects of organophosphate poisoning. *Clinical Neurology and Neurosurgery* 94:93–103.

D'Mello GD, Duffy EA. 1985. The acute toxicity of sarin in marmosets (*Callithrix jacchus*): A behavioral analysis. *Fundamentals in Applied Toxicology* 5(6 Pt 2):S169–S174.

Duncan EJ, Conley JD, Grychowski KD, Conley SA, Lundy PM, Hamilton MG, Sawyer TW. 2001. A comparison of the effects of sarin and succinylcholine on respiratory parameters in anesthetized domestic swine. *Military Medicine* 166(4):322–327.

Ecobichon DJ, Joy RM, eds. 1994. *Pesticides and Neurological Diseases.* 2nd ed. Boca Raton, FL: CRC Press.

Ehrich M, Jortner BS. 2001. Organophosphorous-induced delayed neuropathy. In: Krieger RI, ed. *Handbook of Pesticide Toxicology.* Vol. 2, Agents. 2nd ed. San Diego: Academic Press. Pp. 997–1012.

Eyer P. 1995. Neuropsychopathological changes by organophosphorus compounds—a review. *Human and Experimental Toxicology* 14(11):857–864.

Fernando JC, Hoskins BH, Ho IK. 1984. A striatal serotonergic involvement in the behavioural effects of anticholinesterase organophosphates. *European Journal Pharmacology* 98(1):129–132.

Fidder A, Hulst AG, Noort D, de Ruiter R, van der Schans MJ, Benschop HP, Langenberg JP. 2002. Retrospective detection of exposure to organophosphorus anti-cholinesterases: Mass spectrometric analysis of phosphylated human butyrylcholinesterase. *Chemical Research in Toxicology* 15(4):582–590.

Fonnum F, Sterri SH. 1981. Factors modifying the toxicity of organophosphorous compounds including soman and sarin. *Fundamentals in Applied Toxicology* 1(2):143–147.

Fredriksson SÅ, Hammerström LG, Henriksson L, et al. 1995. Trace determination of alkyl methylphosphonic acids in environmental and biological samples using gas chromatography/negative-ion chemical ionization mass spectrometry and tandem mass spectrometry. *Journal of Mass Spectrometry* 30:1133–1143.

Furlong CE, Costa LG, Hassett C, Richter RJ, Sundstrom JA, Adler DA, Disteche CM, Omiecinski CJ, Chapline C, Crabb JW, Humbert R. 1993. Human and rabbit paraoxonases: Purification, cloning, sequencing, mapping and role of polymorphism in organophosphate detoxification. *Chemical-Biological Interactions* 87:35–48.

Gallo MA, Lawryk NJ. 1991. Organic phosphorus pesticides. In: Hayes WJ Jr, Laws ER Jr, eds. *Handbook of Pesticide Toxicology*. San Diego: Academic Press. Pp. 917–1123.

Goldman M, Klein AK, Kawakami TG, Rosenblatt LS. 1988. *Toxicity Studies on Agents GB (Sarin, Types I and II) and GD (Soman)*. Available from the National Technical Information Service. NTIS AD-A187841.

Goldstein BD, Fincher DR, Searle JR. 1987. Electrophysiological changes in the primary sensory neuron following subchronic soman and sarin: Alterations in sensory receptor function. *Toxicology and Applied Pharmacology* 91(1):55–64.

Gordon J, Inns R, Johnson M, Leadbeater L, Maidment M, Upshall D, Cooper G, Rickard R. 1983. The delayed neuropathic effects of nerve agents and some other organophosphorus compounds. *Archives of Toxicology* 52(2):71–82.

Gray AP. 1984. Design and structure–activity relationships of antidotes to organophosphorus anticholinesterase agents. *Drug Metabolism Reviews* 15(3):557–589.

Grob D. 1963. Anticholinesterase intoxication in man and its treatment. *Handbuch der Experimentellen Pharmakologie* 15(Supplement, Chapter 22):989–1027.

Haley RW, Billecke S, La Du BN. 1999. Association of low PON1 type Q (type A) arylesterase activity with neurologic symptom complexes in Gulf War veterans. *Toxicology and Applied Pharmacology* 157(3):227–233.

Hartgraves SL, Murphy MR. 1992. Behavioral effects of low-dose nerve agents. In: Somani SM, ed. *Chemical Warfare Agents*. San Diego, CA: Academic Press. Pp. 125–154.

He F, Xu H, Qin F, Xu L, Huang J, He X. 1998. Intermediate myasthenia syndrome following acute organophosphates poisoning—an analysis of 21 cases. *Human and Experimental Toxicology* 17(1):40–45.

Henderson RF, Barr EB, Blackwell WB, Clark CR, Conn CA, Kalra R, March TH, Sopori ML, Tesfaigzi Y, Menache MG, Mash DC, Dokladny K, Kozak W, Kozak A, Wachulec M, Rudolph K, Kluger MJ, Singh SP, Razani-Boroujerdi S, Langley RJ. 2001. Response of F344 rats to inhalation of subclinical levels of sarin: Exploring potential causes of Gulf War illness. *Toxicology and Industrial Health* 17(5–10):294–297.

Henderson RF, Barr EB, Blackwell WB, Clark CR, Conn CA, Kalra R, March TH, Sopori ML, Tesfaigzi Y, Menache MG, Mash DC. 2002. Response of rats to low levels of sarin. *Toxicology and Applied Pharmacology* 184(2):67–76.

Hoskins B, Fernando JC, Dulaney MD, Lim DK, Liu DD, Watanabe HK, Ho IK. 1986. Relationship between the neurotoxicities of soman, sarin and tabun, and acetylcholinesterase inhibition. *Toxicology Letters* 30(2):121–129.

Hotopf M, Mackness MI, Nikolaou V, Collier DA, Curtis C, David A, Durrington P, Hull L, Ismail K, Peakman M, Unwin C, Wessely S, Mackness B. 2003. Paraoxonase in Persian Gulf War veterans. *Journal of Occupational and Environmental Medicine* 45(7):668–675.

Husain K, Vijayaraghavan R, Pant SC, Raza SK, Pandey KS. 1993. Delayed neurotoxic effect of sarin in mice after repeated inhalation exposure. *Journal of Applied Toxicology* 13(2):143–145.

Husain K, Pant SC, Raza SK, Singh R, Das Gupta S. 1995. A comparative study of delayed neurotoxicity in hens following repeated administration of organophosphorus compounds. *Indian Journal of Physiology and Pharmacology* 39(1):47–50.

Hulet SW, McDonough JH, Shih TM. 2002. The dose-response effects of repeated subacute sarin exposure on guinea pigs. *Pharmacology, Biochemistry & Behavior* 72(4):835–845.

IOM (Institute of Medicine). 2000. *Gulf War and Health, Volume 1: Depleted Uranium, Pyridostigmine Bromide, Sarin, and Vaccines*. Washington, DC: National Academy Press.

Jacobson KH, Christensen MK, DeArmon IA Jr, Oberst FW. 1959. Studies of chronic exposures of dogs to GB (isopropyl methylphosphonofluoridate) vapor. *Archives of Industrial Health* 19(1):5–10.

Jamal GA. 1997. Neurological syndromes of organophosphorus compounds. *Adverse Drug Reactions and Toxicological Reviews* 16(3):133–170.

Kaloianova FP, El Batawi MA. 1991. *Human Toxicology of Pesticides*. Boca Raton, FL: CRC Press.

Kalra R, Singh SP, Razani-Boroujerdi S, Langley RJ, Blackwell WB, Henderson RF, Sopori ML. 2002. Subclinical doses of the nerve gas sarin impair T cell responses through the autonomic nervous system. *Toxicology and Applied Pharmacology* 184(2):82–87.

Kassa J, Krocova Z, Vachek J. 2000. Long-term alteration of immune functions following low level exposure to sarin in rats. *Acta Medica* 43(3):91–94.

Kassa J, Koupilova M, Vachek J. 2001a. Long-term effects of low-level sarin inhalation exposure on the spatial memory of rats in a T-maze. *Acta Medica* 44(3):93–96.

Kassa J, Koupilova M, Vachek J. 2001b. The influence of low-level sarin inhalation exposure on spatial memory in rats. *Pharmacology, Biochemistry & Behavior* 70(1):175–179.

Kassa J, Pecka M, Tichy M, Bajgar J, Koupilova M, Herink J, Krocova Z. 2001c. Toxic effects of sarin in rats at three months following single or repeated low-level inhalation exposure. *Pharmacology and Toxicology* 88(4):209–212.

Kassa J, Krejcova G, Vachek J. 2002. The impairment of spatial memory following low-level sarin inhalation exposure and antidotal treatment in rats. *Acta Medica (Hradec Kralove)* 45(4):149–153.

Klein AK, Nasr ML, Goldman M. 1987. The effects of in vitro exposure to the neurotoxins sarin (GB) and soman (GD) on unscheduled DNA synthesis by rat hepatocytes. *Toxicology Letters* 38(3):239–249.

Krejcova G, Kassa J, Vachek J. 2002. Effect of atropine and the oxime HI-6 on low-level sarin-induced alteration of performance of rats in a T-maze. *Acta Medica* 45(3):107–110.

LaBorde JB, Bates HK, Dacre JC, Young JF. 1996. Developmental toxicity of sarin in rats and rabbits. *Journal of Toxicology and Environmental Health* 47(3):249–265.

Landauer MR, Romano JA. 1984. Acute behavioral toxicity of the organophosphate sarin in rats. *Neurobehavioral Toxicology and Teratology* 6(3):239–243.

Little PJ, Reynolds ML, Bowman ER, Martin BR. 1986. Tissue disposition of [^{3}H]sarin and its metabolites in mice. *Toxicology and Applied Pharmacology* 83(3):412–419.

Lotti M. 2000. Organophosphorous compounds. In: Spencer P, Schaumburg H, Ludolph A, eds. *Experimental and Clinical Neurotoxicology*. 2nd edition. New York: Oxford University Press. Pp. 897–925.

Lotti M. 2001. Clinical toxicology of anticholinesterase agents in humans. In: Krieger RI, ed. *Handbook of Pesticide Toxicology.* Vol. 2, Agents. 2nd ed. San Diego, CA: Academic Press. Pp. 1043–1085.

Mackness B, Durrington PN, Mackness MI. 2000. Low paraoxonase in Persian Gulf War veterans self-reporting Gulf War Syndrome. *Biochemical and Biophysical Research Communications* 24:729–733.

Marrs TC, Maynard RL, Sidell FR. 1996. *Chemical Warfare Agents: Toxicology and Treatment*. New York: John Wiley & Sons.

Matsuda Y, Nagao M, Takatori T, Niijima H, Nakajima M, Iwase H, Kobayashi M, Iwadate K. 1998. Detection of the sarin hydrolysis product in formalin-fixed brain tissues of victims of the Tokyo subway terrorist attack. *Toxicology and Applied Pharmacology* 150(2):310–320.

Mattsson JL, Wilmer JW, Shankar MR, Berdasco NM, Crissman JW, Maurissen JP, Bond DM. 1996. Single-dose and 13-week repeated-dose neurotoxicity screening studies of chlorpyrifos insecticide. *Food and Chemical Toxicology* 34(4):393–405.

Maurissen JP, Shankar MR, Mattsson JL. 2000. Chlorpyrifos: Lack of cognitive effects in adult Long-Evans rats. *Neurotoxicology and Teratology* 22(2):237–246.

Minami M, Hui DM, Katsumata M, Inagaki H, Boulet CA. 1997. Method for the analysis of the methylphosphonic acid metabolites of sarin and its ethanol-substituted analogue in urine as applied to the victims of the Tokyo sarin disaster. *Journal of Chromatography B Biomedical Sciences and Applications* 695(2):237–244.

Minami M, Hui DM, Wang Z, Katsumata M, Inagaki H, Li Q, Inuzuka S, Mashiko K, Yamamoto Y, Ootsuka T, Boulet CA, Clement JG. 1998. Biological monitoring of metabolites of sarin and its by-products in human urine samples. *Journal of Toxicologic Sciences* 23(Suppl 2):250–254.

Moore DH. 1998. Long term health effects of low dose exposure to nerve agent. *Journal of Physiology* 92(3–4):325–328.

Nagao M, Takatori T, Matsuda Y, Nakajima M, Iwase H, Iwadate K. 1997. Definitive evidence for the acute sarin poisoning diagnosis in the Tokyo subway. *Toxicology and Applied Pharmacology* 144(1):198–203.

Nieminen SA, Lecklin A, Heikkinen O, Ylitalo P. 1990. Acute behavioural effects of the organophosphates sarin and soman in rats. *Pharmacology and Toxicology* 67(1):36–40.

Niijima H, Nagao M, Nakajima M, Takatori T, Matsuda Y, Iwase H, Kobayashi M. 1999. Sarin-like and soman-like organophosphorous agents activate PLC gamma in rat brains. *Toxicology and Applied Pharmacology* 156(1):64–69.

Niijima H, Nagao M, Nakajima M, Takatori T, Iwasa M, Maeno Y, Koyama H, Isobe I. 2000. The effects of sarin-like and soman-like organophosphorus agents on MAPK and JNK in rat brains. *Forensic Science International* 112(2–3):171–178.

Noort D, Hulst AG, Platenburg DH, Polhuijs M, Benschop HP. 1998. Quantitative analysis of O-isopropyl methylphosphonic acid in serum samples of Japanese citizens allegedly exposed to sarin: Estimation of internal dosage. *Archives of Toxicology* 72(10):671–675.

NRC (National Research Council). 2003. *Acute Exposure Guideline Levels for Selected Airborne Chemicals: Volume 3*. Washington DC: The National Academies Press.

Pearce PC, Crofts HS, Muggleton NG, Ridout D, Scott EAM. 1999. The effects of acutely administered low dose sarin on cognitive behaviour and the electroencephalogram in the common marmoset. *Journal of Psychopharmacology* 13(2):128–135.

Polhuijs M, Langenberg JP, Benschop HP. 1997. New method for retrospective detection of exposure to organophosphorus anticholinesterases: Application to alleged sarin victims of Japanese terrorists. *Toxicology and Applied Pharmacology* 146(1):156–161.

Rickett DL, Glenn JF, Beers ET. 1986. Central respiratory effects versus neuromuscular actions of nerve agents. *Neurotoxicology* 7(1):225–236.

Rocha ES, Chebabo SR, Santos MD, Aracava Y, Albuquerque EX. 1998. An analysis of low level doses of cholinesterase inhibitors in cultured neurons and hippocampal slices of rats. *Drug and Chemical Toxicology* 21 (Suppl 1):191–200.

Shailesh KK, Pais P, Vengamma B, Muthane U. 1994. Clinical and electrophysiological study of intermediate syndrome in patients with organophosphorous poisoning. *Journal of the Association of Physicians of India* 42(6):451–453.

Shih ML, Smith JR, McMonagle JD, Dolzine TW, Gresham VC. 1991. Detection of metabolites of toxic alkylmethylphosphonates in biological samples. *Biological Mass Spectrometry* 20(11):717–723.

Shih ML, McMonagle JD, Dolzine TW, Gresham VC. 1994. Metabolite pharmacokinetics of soman, sarin and GF in rats and biological monitoring of exposure to toxic organophosphorus agents. *Journal of Applied Toxicology* 14(3):195–199.

Sidell FR, Borak J. 1992. Chemical warfare agents: II. Nerve agents. *Annals of Emergency Medicine* 21(7):865–871.

Singer AW, Jaax NK, Graham JS, McLeod CG Jr. 1987. Cardiomyopathy in soman and sarin intoxicated rats. *Toxicology Letters* 36(3):243–249.

Somani SM. 1992. *Chemical Warfare Agents*. New York: Academic Press.

Spencer P, Wilson B, Albuquerque E. 2000. Sarin, other "nerve agents," and their antidotes. In: Spencer P, Schaumburg H, Ludolph A, eds. *Experimental and Clinical Neurotoxicology*. 2nd edition. New York: Oxford University Press.

Spruit HE, Langenberg JP, Trap HC, van der Wiel HJ, Helmich RB, van Helden HP, Benschop HP. 2000. Intravenous and inhalation toxicokinetics of sarin stereoisomers in atropinized guinea pigs. *Toxicology and Applied Pharmacology* 169(3):249–254.

Stewart CE, Sullivan J Jr. 1992. Military munitions and antipersonnel agents. In: Sullivan JB Jr, Krieger G, eds. *Hazardous Materials Toxicology: Clinical Principles of Environmental Health*. Baltimore, MD: Williams & Wilkins. Pp. 986–1014.

Tørnes JA. 1996. Identification of alkyl methylphosphonic acids by thermospray tandem mass spectrometry. *Rapid Communications in Mass Spectrometry* 10:878–882.

Tripathi HL, Dewey WL. 1989. Comparison of the effects of diisopropylfluorophosphate, sarin, soman, and tabun on toxicity and brain acetylcholinesterase activity in mice. *Journal of Toxicology and Environmental Health* 26(4):437–446.

Willems JL, Palate BM, Vranken MA, De Bisschop HC. 1983. *Proceedings of the International Symposium on Protection against Chemical Warfare Agents.* Umea, Sweden: National Defense Research Institute. Pp. 95–100.

Yamada Y, Takatori T, Nagao M, Iwase H, Kuroda N, Yanagida J, Shinozuka T. 2001. Expression of paraoxonase isoform did not confer protection from acute sarin poisoning in the Tokyo subway terrorist attack. *International Journal of Legal Medicine* 115(2):82–84.

Yamasaki Y, Sakamoto K, Watada H, Kajimoto Y, Hori M. 1997. The Arg192 isoform of paraoxonase with low sarin-hydrolyzing activity is dominant in the Japanese. *Human Genetics* 101(1):67–68.

3

Human Health Outcome Studies

This chapter focuses on epidemiologic studies that were not reviewed in *Gulf War and Health: Volume 1*, hereafter referred to as *GW1* (IOM, 2000). Four populations have been studied in large epidemiologic studies after exposure to sarin: military volunteers who were exposed several decades ago to nonlethal doses of sarin and other chemical-warfare agents, industrial workers with documented acute exposure to sarin, victims of the sarin terrorist attacks in Matsumoto City in 1994 and Tokyo in 1995, and Gulf War veterans. Studies of Gulf War veterans include studies of veterans potentially exposed to sarin after demolition of rockets at Khamisiyah, Iraq, and a number of studies that evaluate the relationship between symptoms and possible exposures, including to sarin or cyclosarin, on the basis of a self-reporting questionnaire. Studies reviewed in *GW1* (IOM, 2000) are briefly reviewed to provide a complete picture of the available data. This chapter reviews and critiques those studies. The data from those studies are summarized by health outcome in Chapter 4.

A major limitation of most human studies of the health effects of sarin is a lack of exposure information. Most studies of sarin were undertaken after occupational accidents or terrorist attacks, and the magnitudes of exposures can only be inferred from clinical effects. High exposure is inferred from the presence of the acute cholinergic syndrome (see Chapter 2 for description) that requires hospitalization or emergency treatment. Intermediate exposure is inferred from minimal or threshold cholinergic effects (miosis or rhinorrhea) and a small decrease (less than 20%) in blood cholinesterase activity. Low exposure is inferred from proximity to a documented exposure and the absence of clinically detectable

cholinergic signs or symptoms or detectable change in blood cholinesterase activity (Brown and Brix, 1998).

Although there were no medical reports at the time of the release at Khamisiyah that were consistent with the signs and symptoms of acute exposure to sarin (PAC, 1996), there is concern that exposure of US troops during the Gulf War might have occurred. The level of exposure would have been insufficient to produce the acute cholinergic syndrome. Therefore, this chapter reviews available human studies, focusing mainly on epidemiologic studies of the long-term health effects of sarin, and is organized by study population.

Relatively few studies have looked at the long-term health effects of low-dose exposure to sarin or cyclosarin, but a number of epidemiologic studies have been conducted on organophosphorus (OP) insecticides, which have some common mechanisms of action. The health effects of those insecticides are reviewed in *Gulf War and Health: Volume 2* (IOM, 2003), and the conclusions of that report and epidemiologic studies that have been published since that report was prepared are discussed in Appendix A.

INTENTIONALLY EXPOSED MILITARY VOLUNTEERS

In the past, military authorities (including those of the US and the UK) have conducted dosing studies of chemicals in healthy servicemen who volunteer after being informed of the protocol and risks involved. Although questions remain regarding the ethics of such studies and their ethical acceptability by current standards, such studies have been conducted in the past with servicemen voluntarily exposed to sarin and other chemical-warfare agents. The studies are reviewed in this section, and those published since the preparation of *GW1* are summarized in Table 3-1.

US Military Studies

From 1958 to 1975, the US Army studied nearly 7,000 servicemen who had voluntarily agreed to be exposed to an array of chemical-warfare agents at Edgewood Arsenal, Maryland. At the request of the Army, the Medical Follow-up Agency (MFUA; now part of the Institute of Medicine) of the National Research Council designed and conducted two studies of the long-term health effects of those exposures. The first, a followup at least 10 years after exposure (NRC, 1985), was described in *GW1* (IOM, 2000). The study examined current health status and hospital admissions to military or Veterans' Administration hospitals. In that survey, the subgroup exposed to a variety of anticholinesterases as a class had no long-term health consequences of exposure, but the study had low statistical power and other methodologic problems acknowledged by the authors.

Since the preparation of *GW1* (IOM, 2000), MFUA conducted a second

study using a survey almost identical with the 1985 one (Page, 2003). The study, which was conducted at least 25 years after the end of the testing program, included a mortality study and a telephone survey of 4,022 members of the program. The survey was designed to assess neuropsychologic, neurologic, and vestibular symptoms possibly related to anticholinesterase exposure in the three exposure groups: a group exposed to anticholinesterase, a group exposed to two or more nonanticholinesterase agents (such as, scopolamine and atropine), and a nonexposed group that at the program's inception was ineligible for participation because of low scores on general intelligence tests and the Minnesota Multiphasic Personality Inventory. The anticholinesterase-exposed group was exposed to at least one of 15 anticholinesterase agents, the most common being Agent VX (n = 740), sarin (n = 246), and eserine (physostigmine, n = 138). Exact doses are not known, but in an appendix to the 2003 study, the authors note that their review of original records found 17 of 25 sarin-exposed servicemen to have experienced the acute cholinergic syndrome. The authors were unable to assemble a sarin-only group because three-fourths of the original sample of 246 either had died (n = 67) or had unusable dose data. As noted earlier, a mortality study was also conducted.

There were no statistically significant differences among the three groups in overall health, disability, reproductive history, and psychologic symptoms after adjustment for age, initial fitness, race, and chemical exposures outside the program. However, MFUA believed that, because of selection bias, the nonexposed group was less healthy than the anticholinesterase-exposed group, whereas the nonanticholinesterase group was likely to be healthier. Only sleep disturbances were more prevalent in the anticholinesterase group than in the nonexposed group (sleep disturbances score, 0–9; mean difference, +0.28; 95% CI did not include 1). Attention problems were reported less frequently in the anticholinesterase group than in the nonanticholinesterase group. There was, however, significantly lower all-cause mortality in the anticholinesterase group than in the nonexposed group (relative risk [RR] for all-cause mortality, 0.82; 95% CI, 0.68–0.99). There were no mortality differences for specific conditions, such as suicide, accidental deaths, cancer, and heart disease. Although the results of the study show no association, it is somewhat uninformative for the purpose of this committee because of the lack of dose information, the fact that the nonexposed control group was likely to be less healthy, and the mixed nature of the exposures analyzed (there was no analysis of a sarin-only group).

UK Military Studies

In an uncontrolled study of UK servicemen who volunteered to be exposed to sarin (sarin vapors at 15 mg/min-m^3, in 1983–1984) and displayed some signs of the acute cholinergic syndrome (Baker and Sedgwick, 1996; discussed in IOM, 2000), the authors interpreted an increased jitter 3 h after exposure and still

TABLE 3-1 Non-Gulf War Veteran Studies of Sarin Not Discussed in *Gulf War and Health: Volume 1*

Reference	Type of Study and Study Population	Exposure Determination	Health Outcome, and How and When Measured
Page, 2003	Follow-up study of military volunteers for 1955–1975 Edgewood, Maryland, program; one group with anticholinesterase exposure (n = 1,339) vs exposed to two or more nonanticholinesterase agents (n = 1,359) vs no chemical test (nonexposed) (n = 1,324)	Military deliberately administered 250 agents, including sarin, cyclosarin, and 13 other anticholinesterases; doses not carefully recorded; sarin doses may have ranged from 3.0 to 4.0 μg/kg	Mortality records from VA and Social Security Administration, survey of neuropsychologic impairment, illness attitudes, peripheral nerve disease, vestibular dysfunction, sleep disorders, and reproductive history. Surveys conducted at least 25 years after exposures
Nishiwaki et al., 2001	56 exposed rescue workers and police officers vs 52 nonexposed matched controls in same departments	High- and low-exposure group from self-reports of hospitalizations vs outpatient treatment	Five neurobehavioral tests, stabilometry, vibration perception, and IES-R-J and general health questionnaire conducted 3 years after exposure
Kawana et al., 2001	Follow-up of 582 patients treated at St. Luke's hospital in Toyko at 2, 3, and 5 years No control group	Not clear from study	33-item mailed questionnaire at three times (1997, 1998, 2000; 2, 3, and 5 years after exposure) covering physical and psychologic symptoms related to sarin; PTSD assessed three ways

Abbreviations: IES-R-J, Impact of Event Scale; PTSD, posttraumatic stress disorder; SES, socioeconomic status.

Results	Adjusted RR or OR (95% CI or *p*)	Limitations
No excess mortality from particular conditions, but less mortality from all causes in anticholinesterase-exposed than unexposed; fewer attention problems in anticholinesterase-exposed vs other warfare agents; greater sleep disturbances than nonexposed	RR for all-cause mortality 0.89 in anticholinesterase-exposed vs nonexposed (95% CI, 0.68–0.99)	Lack of dose information and inability to assemble a sarin-only cohort
Dose–effect relationship with backward digit span memory performance, using multiple logistic regression, and findings independent of trauma symptoms; adjusted tapping interval for dominant hand worse in high-exposed group than controls; stabilometry measures with eyes open significantly worse in low-exposed group than controls, but no dose effect	Backward digit span: high-dose adjusted OR, 3.19 (95% CI, 1.06–10.38) and low-dose OR, 1.17 (95% CI, 0.42–3.23)	Not clear whether medical-record check conducted to verify self-reported level of exposure
Most-frequent symptoms: eye symptoms (tiredness of eyes, dim vision, difficulty focusing), tiredness, fatigue, stiff muscles, headache, depressed mood; prevalence (1997, 1998, 2000): DSM-IV PTSD (2.8, 2.9, 2.1%); partial PTSD (7.1, 7.3, 8.4%), PTSD–Nakano (12.4, 9.7, 14.1%)		No control group, low response rate, methods of dose determination or subject selection not reported

apparent 1 year but not 2 years after exposure as a possible indicator of the intermediate syndrome (see Chapter 2). (Jitter is a variation in time of onset of second action potential within motor unit after initial discharge. It is one indication of potential failure of transmission at the neuromuscular junction.) The intermediate syndrome itself did not occur.

No studies of UK veterans have been published since the preparation of *GW1*.

ACCIDENTALLY EXPOSED INDUSTRIAL WORKERS

In the first controlled study of long-term central nervous system (CNS) effects in workers accidentally exposed to sarin, researchers compared electroencephalographic (EEG) activity in workers at a manufacturing plant known to have been accidentally exposed to sarin (n = 77) with activity in nonexposed controls at the same plant (n = 38) (Duffy et al., 1979; Burchfiel and Duffy, 1982). Exposed workers had not been exposed within a year of the study, but had had one or more exposure incidents (clinical signs and at least a 25% inhibition of red-cell cholinesterase activity) within the previous 6 years. Although some differences in EEG results were seen, the clinical significance of the changes was not clear. Exposed workers also reported increased dreaming, instances of irritability, disturbed memory, and difficulty in maintaining alertness and attention (Burchfiel and Duffy, 1982), but methodologic details of symptom reporting were not provided.

No studies of people accidentally exposed to sarin in industrial accidents have been published since the preparation of *GW1*.

JAPANESE TERRORIST ATTACKS

Matsumoto

In 1994, Japanese terrorists spread sarin vapor with a heater and fan mounted on a truck in a residential neighborhood near the center of Matsumoto, Japan (Nakajima et al., 1997). About 600 people (residents and rescue teams) developed the acute cholinergic syndrome; 253 sought medical assistance, 58 were admitted to hospitals, and 7 died. Several case reports, case series, and a population-based epidemiologic study conducted after that attack were described in *GW1* (IOM, 2000). One of the case series found that four of six severely poisoned patients displayed visual-field defects, hypoxia, low-grade fever, and what were described as "epileptic electroencephalographic changes" up to 2 years after exposure (Sekijima et al., 1997). At 7 months after exposure, one patient also developed sensory polyneuropathy and reduced sensory-nerve conduction velocity, but the characteristics are not consistent with classic organophosphate-induced delayed neuropathy (OPIDN) (IOM, 2000). The population-based study

used mailed questionnaires and identified symptoms that persisted up to 3 years after exposure (odds ratios were highest for fatigue, headache, and the visual disturbances asthenopia, blurred vision, and narrowing of visual field) among those closest to the site of sarin release (Nakajima et al., 1998, 1999).

Since the preparation of *GW1*, a non-English-language study with an English abstract (Nohara, 1999) had reported detailed ophthalmologic tests, EEG results, and electrocardiographic (ECG) test results in people living close to the site of sarin release 45 months after the attack. The abstract does not state the number of subjects or how cases were ascertained. Findings reported in the abstract include visual-field constriction, posttraumatic stress disorder (PTSD), and abnormal EEG and ECG readings up to 45 months after the attack.

Tokyo

On March 20, 1995, terrorists released diluted sarin vapor simultaneously into three converging lines of the Tokyo subway system. About 1,000 people were symptomatic after the attack, and 12 died. *GW1* (IOM, 2000) described in detail the findings of several epidemiologic studies conducted months after the attack. Those studies were of patients who had been seen at the hospital that treated the largest number of patients (n = 641), St. Luke's International. About 83% had intermediate exposure and 17% had high exposure, on the basis of symptom profiles and as verified by more than a 20% decrease in blood cholinesterase activity. Detailed neurophysiologic and neuropsychologic testing was conducted several months later on 18 symptom-free survivors with previous intermediate or high exposure. By symptom-free, the authors meant absence of obvious ophthalmologic, cardiovascular, neurologic or other confounding disorders, such as ischemic heart disease, multiple sclerosis, diabetes, or alcohol dependence. Cholinesterase, measured in 13 of the 18 survivors, had returned to normal. The studies described in *GW1* (IOM, 2000) found the 18 to have significantly more symptoms of PTSD, impaired performance on one (the digit-symbol test) of nine neurobehavioral tests, and significant changes in event-related potential, visual-evoked potential, and postural sway testing (Murata et al., 1997; Yokoyama et al., 1998a,b,c). Studies published since the preparation of *GW1* (IOM, 2000) are summarized in Table 3-1 and are discussed below.

Yokoyama et al. (2002) have published further information on the health of survivors. The investigators previously had reported, in a study evaluated in *GW1*, that 6–8 months after the attacks female (n = 9), but not male, survivors with confirmed intermediate or high exposure had abnormal findings on computerized posturography (Yokoyama et al, 1998a). The more recent study compares the computerized posturography data on the nine sarin-poisoned females and matched controls of the earlier study with those on cohorts exposed to lead or solvents to clarify which of the cerebellar pathways is most affected. This study, however, is not as useful for determining the effects of sarin, in that it reports

only the results in the sarin-poisoned females that were previously reported (Yokoyama et al., 1998a).

Nishiwaki et al. (2001) compared neurophysiologic performance 3 years after the Tokyo attack in 56 exposed men in rescue teams (fire department rescue workers and police) with that in 52 nonexposed controls matched for age and occupation in the same departments. Rescue workers responding at the site were unaware of the sarin release and did not wear protective gear. Exposed workers were divided into high-exposure and low-exposure groups, on the basis of self-reports of hospitalization or outpatient treatment, respectively. Although the authors report that they "check[ed] the self administered questionnaire", it is unclear from the publication whether they used medical records to verify self-reports about the site of treatment. Exposed and nonexposed groups were evaluated with five neurobehavioral tests (finger-tapping test for dominant and non-dominant hands, simple reaction time, choice reaction time, backward digit span, and Benton Visual Retention), stabilometry, vibration perception, and two questionnaires (the Japanese-language version of the Impact of Events Scale-Revised (IES-R-J) and the General Health Questionnaire). With multiple logistic regression, a dose-effect relationship was found for the backward-digit-span test, which is a test of attention and concentration. The mean maximal digit number was 4.24 ± 0.72 and 4.69 ± 1.37 in the high- and low-exposure group, respectively. Those results, however, do not appear to be of clinical significance. The finding was independent of trauma symptoms from the IES-R-J. Adjusted tapping interval for dominant hand was worse in the high-exposure group than in controls, and stabilometry measures with eyes open were significantly worse in the low-exposure group than in controls, but no dose-effect relationship was found for those end points. Scores on the IES-R-J and the General Health Questionnaire were higher in both exposed groups than in controls, but the difference was not statistically significant. Although the results of the study are intriguing, the result of only one test or a small number of tests (three) was affected, and further study of neurophysiologic performance is needed.

Kawana et al. (2001) reports on results of a 33-item questionnaire of physical and psychologic symptoms mailed at three times—1997, 1998, and 2000—to 582 patients who had been treated at St. Luke's International Hospital on the day of the sarin attack. The relationship of this cohort to a cohort of 610 patients surveyed by the same hospital 1, 3, and 6 months after the attack (Ohbu et al., 1997) is unclear. According to Kawana et al. (2001), the Ohbu et al. (1997) study found 60% of respondents reporting symptoms of PTSD. Kawana et al. (2001) did not have a control population, although it did compare its findings on PTSD with those from other studies of Tokyo and Matsumoto sarin attacks, and the response rate was low (49% in 1997, 35% in 1998, and 32.8% in 2000). The investigators applied symptom-based criteria for PTSD with three sets of criteria: DSM-IV, "partial PTSD" (one symptom from each PTSD symptom cat-

egory of avoidance, hyperarousal, and re-experience), and a new method, "PTSD-Nakano", named after the Japanese researcher who revised PTSD diagnostic criteria "to require at least one physical symptom in addition to one avoidance symptom, and one hyperarousal or one re-experiencing symptom." No validation or reference is provided for the third category.

The most frequently reported symptoms, reported by more than 15% of respondents in all years, were ocular symptoms (tiredness of eyes, dim vision, and difficulty focusing), tiredness, fatigue, stiff muscles, and headache. Depressed mood was reported by 13%, 24%, and 17% of respondents in 1997, 1998, 2000, respectively—showing a significant change over time. Depressed mood was one of the few symptoms that changed significantly over the 5-year period. The prevalence of symptoms was comparable with rates reported in other studies of Japanese survivors of sarin attacks. The prevalence of PTSD (DSM-IV), partial PTSD, and PTSD-Nakano ranged from 2.1–2.9%, 7.1–8.4%, and 9.7–14.1%, respectively, depending on the questionnaire year.

Asukai et al. (2002) studied the reliability and validity of a Japanese-language version of the IES-R-J. That scale has been validated in the United States for assessing magnitude of traumatic-stress exposure for a potential PTSD diagnosis, but little such research has been conducted in non-Western countries. The scale contains 22 items grouped into clusters of symptoms for diagnosing PTSD (hyperarousal, avoidance, and intrusion). Survivors of the Tokyo sarin attack made up one of four groups (workers with lifetime mixed traumatic events, survivors of an arsenic poisoning case, and survivors of the Hanshin-Awaji earthquake made up the other three) in which the new instrument was studied. It is not clear how many people were sent the survey, but investigators report that 658 survivors replied, a substantial number of whom were litigants (the exact number was not reported). The year of the survey was not reported, but a later book chapter by the authors states that the survey was conducted 5 years after the Tokyo attack (Asukai and Maekawa, 2002). The prevalence of high scorers on the IES-R-J in the Tokyo group was 24.6% of males and 35.8% of females, which are within the range of the other three Japanese groups studied. Two of those other groups were also given clinical examinations with the PTSD module of the Structural Clinical Interview for DSM-III-R and the Clinical Administered PTSD Scale. In those clinical evaluations, PTSD was diagnosed in 9–16% of subjects.

GULF WAR VETERANS

After the Gulf War, veterans reported higher rates of fatigue, headache, pain, and cognitive symptoms than did nondeployed military personnel, according to numerous population-based studies in the United States (Iowa Persian Gulf Study Group, 1997; Kang et al., 2000), the United Kingdom (Unwin et al., 1999; Cherry et al., 2001), Canada (Goss Gilroy Inc., 1998), and Denmark (Suadicani et al., 1999). Veterans' symptoms or symptom clusters have been characterized as "un-

explained illnesses" because they do not fit established diagnoses. Health outcomes in the studies were not restricted to established diagnoses, they could be symptoms, sets of symptoms, syndromes, or diagnoses.

More than 20 exposures in the Gulf War, from vaccines to nerve agents, have been studied in relation to veterans' symptoms. This section examines a set of those exposures that are relevant to sarin. Specifically, it examines the nature and quality of the evidence regarding associations between *any* health effect in veterans and *any* possible exposure related to sarin, including nerve agents at Khamisiyah, hearing of chemical-weapon alarms, and wearing of chemical-protection gear. Most studies summarized here have been evaluated in previous IOM volumes because they covered multiple exposures (IOM, 2000, 2003); because of their mandates, past IOM committees' evaluations focused on separate exposures.

Most studies of Gulf War veterans were designed to detect the nature and prevalence of veterans' symptoms and illnesses and whether they constituted a new syndrome rather than specifically to assess the effects of exposure to particular agents of interest. When the effects of exposure to various agents were assessed, numerous potential agents were evaluated in the same study.

Most of the veteran health studies were cross-sectional and were conducted years after the war. Cross-sectional studies limit opportunities to learn about symptom duration and latency of onset; outcomes and exposure to various agents were measured simultaneously after the Gulf War had ended (IOM, 2000). Most Gulf War studies relied on self-reports of exposure, and in most cases the self-reports came years after the end of the war. Most studies did not identify specific environmental agents. Because the studies used self-reported data generally gathered years after the events in question, there is a strong possibility of recall bias—that is, veterans with symptoms would be more likely than those without symptoms to recall exposure. Only one cohort was studied soon after the war and then longitudinally (Proctor et al., 1998). Further, self-reports can be unreliable. A study by McCauley et al. (1999) found low test–retest reliability for the belief in exposure to chemical warfare when veterans responded to the same exposure questions 3 months apart.

In addition, symptoms reported in cross-sectional studies do not necessarily represent accurately the total symptom experience after an exposure. Most of the studies relied on symptom self-reports elicited via questionnaire or structured interview. Several approaches were taken to combine reported symptoms into outcome variables. One approach was to use factor analysis to uncover an underlying structure in reported symptoms (Haley and Kurt, 1997; Fukuda et al., 1998; Cherry et al., 2001). A second approach attempted to match symptoms in some way to previously defined syndromes or illnesses (Iowa Persian Gulf Study Group, 1997; Unwin et al., 1999; Nisenbaum et al., 2000). In some cases, previously validated instruments were used. In others, symptoms were assembled into established syndromes on the basis of criteria devised by the investigators; sub-

jects who did not meet the criteria of established syndromes or diagnoses were said to have nonexplained symptoms that could be related to a Gulf War exposure. Other studies did not attempt a synthesis of any sort but searched for associations between exposures to various agents during the Gulf War and individual symptoms.

Another limitation of Gulf War studies was the problem of multiple comparisons between exposure to numerous agents and health outcomes. When investigators examine a large number of exposure–symptom associations, the chances of reporting a spurious association as statistically significant (a type I error) are increased. Some Gulf War studies took a wide variety of statistical approaches to adjust for the problem of multiple comparisons. However, many did not account for the problem and reported as statistically significant any association with a p value of 0.05 or less. In some of those studies, the investigators did not adjust for multiple comparisons because of the exploratory nature of the study and because of their desire to reduce the probability of not finding a true association (a type II error). Other investigators were more conservative and set a more stringent significance level to reduce the probability of a type I error (Haley and Kurt, 1997; Cherry et al., 2001; White et al., 2001).

Many studies noted that many agents were associated with the outcomes they measured, but only one attempted to examine the association between specific agents and found them to be strongly correlated (Cherry et al., 2001). The remainder of this section discusses the studies of Gulf War veterans that are relevant to sarin exposure. The discussion is divided into two parts: a discussion of studies on troops who were determined, because of their unit locations, to have been potentially exposed to sarin after the weapons demolition at Khamisiyah; and a discussion of studies that examine a number or symptoms of health outcomes in relation to numerous self-reported exposures. Those studies are summarized in Table 3-2.

Studies of Veterans Potentially Exposed at Khamisiyah

As discussed in Chapter 1, it is estimated that almost 100,000 US troops were potentially exposed to low concentrations of sarin and cyclosarin released from the US military demolition of hundreds of rockets at Khamisiyah, Iraq, on March 10, 1991. Troops performing demolitions were unaware of the presence of nerve agent because their alarms, which are sensitive to lethal or near-lethal concentrations of sarin, did not sound. The total amount of chemicals released, according to the most recently published estimates, is 371 kg of sarin and cyclosarin (Winkenwerder, 2002). None of the troops had the acute cholinergic syndrome, according to the US Army Medical Corps and a later survey of 20,000 veterans, but the possibility of low, asymptomatic exposures cannot be discounted. Different methods have been used to estimate possible troop exposures to sarin. Initially, approximately 100,000 soldiers were notified that they might

TABLE 3-2 Studies of Gulf War Veterans Potentially Relevant to Sarin Exposures

Reference	Population	Exposure Assessment for Relevant Agents
Studies of Veterans Potentially Exposed at Khamisiyah Not Reviewed in *GW1*		
Smith et al., 2003	431,762 active-duty US military deployed to Gulf War divided into two groups: Nonexposed (n = 318,458) Possibly exposed (n = 99,614). Active-duty includes all active duty up to 10 years after the war (until separation) and reserve only while on active-duty status (follow-up to Gray et al., 1999)	Second exposure model by DOD of nerve agent release data, meteorological models, and atmospheric removal mechanisms combined with troop positions
Gray et al., 1999	349,291 active-duty US military deployed to Gulf War divided into three groups: Not exposed (n = 224,804) Uncertain low dose exposure (n = 75,717) Estimated subclinical exposure (n = 48,770)	First exposure model by DOD of nerve agent release data, meteorological models combined with troop positions
McCauley et al., 2002	923 Khamisiyah-exposed US Gulf War veterans vs 927 Khamisiyah nonexposed Gulf War veterans vs 1,369 non-Gulf War-deployed veterans from Oregon, Washington, California, Georgia, or North Carolina	Exposure defined by DOD as troop location within a 50-kilometer radius of Khamisiyah

Timeframe and Health-Outcome Assessment	Results	Limitations
DOD hospitalizations (1991–2000) for any cause, diagnoses from 15 categories and specific diagnoses proposed by expert panel	Using Cox modeling, 2 of 37 models showed an increase adjusted risk of hospitalization for cardiac dysrhythmias, circulatory system diseases (RR, 1.07; 95% CI, 1.0–1.13), specifically for cardiac dysrhythmias (RR, 1.23; 95% CI, 1.04–1.44)	Limited to DOD hospitals. Hospitalization data available for only active and reserve Gulf War veterans who remained on active duty or retired with medical benefits after the end of the war. No outpatient data available
DOD hospitalizations (1991–1995) for any cause, diagnoses from 15 categories and specific diagnoses proposed by expert panel	Using Cox modeling, none of the models suggested a dose–response relation or neurologic sequelae	Limited to DOD hospitals. Hospitalization data available for only active and reserve Gulf War veterans who remained on active duty or retired with medical benefits after the end of the war. No outpatient data available. No adjustment for confounding exposures
Computer-assisted telephone interview about Khamisiyah-related exposures, medical conditions diagnosed by a physician, hospitalizations, and disability; interview conducted 8 years after Khamisiyah demolition	No differences between Khamisiyah-exposed and Khamisiyah-nonexposed Gulf War veterans in health conditions. Deployed troops significantly more likely than nondeployed troops to report physician-diagnosed high blood pressure (OR,1.7; 95% CI, 1.3–2.4), heart disease (OR, 2.5; 95% CI, 1.1–6.6), slipped disk or pinched nerve (OR, 1.5; 95% CI, 1.1–2.0), PTSD (OR, 14.9; 95% CI 5.6–60.9), hospitalization for depression (OR, 5.1; 95% CI, 1.5–32.1), periodontal disease (OR, 1.8; 95% CI, 1.2–2.8)	Self-reported conditions recalled 9 yrs after exposure, DOD's models of nerve agent exposure not yet available, not representative of entire Gulf War cohort

continued

TABLE 3-2 Continued

Reference	Population	Exposure Assessment for Relevant Agents
McCauley et al., 2001	2,918 veterans from Oregon, Washington, California, Georgia, or North Carolina	Exposure defined by DOD as troop location within a 50-kilometer radius of Khamisiyah
Self-Reported Exposures: Population-Based Studies		
New Studies		
Reid et al., 2001	Subgroups of UK veterans meeting case criteria for MCS and CFS (same cohort as Unwin et al., 1999)	Three relevant environmental exposures: "NBC suits", "hear chemical alarms", "chemical/nerve gas attack"
Suadicani et al., 1999	686 Gulf War-deployed peacekeepers vs matched controls from Danish armed forces	One relevant exposure: "nerve gas"
Ishoy et al., 1999a	686 Gulf War-deployed peacekeepers vs matched controls from Danish armed forces (same cohort as Suadicani et al., 1999)	One relevant exposure: "nerve gas"

Timeframe and Health-Outcome Assessment	Results	Limitations
Computer-assisted telephone interview about Khamisiyah-related exposures, 24-item symptom checklist during Khamisiyah operations, and current symptom checklist	No significant differences between Khamisiyah-exposed vs nonexposed in current or past symptoms. Numerous significant differences between Khamisiyah-witnesses versus nonwitnesses in past and current symptom reporting. Current symptoms in Khamisiyah-witnesses vs nonwitness: tingling or burning sensations of the skin (OR, 1.7; 95% CI, 1.1–2.8), changes in memory (OR, 1.7; 95% CI, 1.2–2.4), difficulty sleeping (OR, 2.0; 95% CI, 1.2–3.5), persistent fatigue (OR, 1.8; 95% CI, 1.2–2.6), depression (OR, 1.6; 95% CI, 1.1–2.4), and bloody diarrhea (OR, 3.1; 95% CI, 1.6–6.0)	Self-reported symptoms recalled 9 yrs after exposure, DOD's models of nerve agent exposure not yet available, not representative of entire Gulf War cohort
Symptom questionnaires, exposure questionnaire, both 6–7 years after Khamisiyah demolition	In Gulf War veterans, MCS associated with "hear chemical alarms" (OR, 2.5; 95%, CI 1.0–5.9), "chemical/ nerve gas attack" (OR, 4.6; 95% CI, 1.6–13.3), CFS associated with "hear chemical alarms" (OR, 2.5; 95% CI, 1.2–5.3)	Self-reported symptoms and exposures
Symptom questionnaires, exposure questionnaire up to 6 years after return	In Gulf War cohort, "nerve gas" not significantly associated with neuropsychologic symptoms	Self-reported symptoms and exposures
Symptom questionnaires (GI symptoms), exposure questionnaire, clinical examination up to 6 years after return	After multivariate adjustment, nerve gas not significantly associated with GI symptoms	Self-reported symptoms and exposures

continued

TABLE 3-2 Continued

Reference	Population	Exposure Assessment for Relevant Agents
Spencer et al., 2001	Random sample (n = 2,343) of 23,711 Gulf War veterans from Oregon or Washington state, nested case–control study	Three relevant exposures: "chemical decontamination bottles", "inadequate protection during chemical/SCUD alarms", "worked around chemical warfare agents"
Kang et al., 2002	11,441 US veterans deployed to Gulf War vs 9,476 non–Gulf War-deployed, nested case–control study	One relevant exposure: "nerve gas"
Kang et al., 2003	11,441 US veterans deployed to Gulf War vs 9,476 non–Gulf War-deployed, nested case–control study	"Had worn chemical protective gear or heard chemical alarms sounding" was one of three combat stressors; other combat stressors: "had been involved in direct combat duty" and "had witnessed any deaths"

Timeframe and Health-Outcome Assessment	Results	Limitations
Exposure questionnaire, symptom questionnaires collected 4–7 years after Khamisiyah demolition, clinical examination to verify case of unexplained illness	By simple logistic regression, cases of unexplained illness (n = 241) more likely than healthy Gulf War-deployed controls (n = 113) to report "inadequate protection during chemical/SCUD alarm"	Self-reported symptoms and exposures, multivariate analysis not performed on exposures of interest
Potentially new neurologic syndrome via factor analysis, symptom questionnaires, exposure questionnaire, surveys conducted in 1995	"Nerve gas" among nine self-reported exposures at least three times more common in 277 Gulf War-deployed veterans (cases) with these symptoms (loss of balance or dizziness, speech difficulty, sudden loss of strength, tremors or shaking) than in Gulf War-deployed noncases (42.3% of cases reported nerve gas exposure vs 4.6% of Gulf War-deployed noncases)	Self-reported symptoms and exposures, no analysis for dose–response relationship
PTSD, CFS; surveys conducted from 1995–1997	PTSD excess (adjusted OR, 3.1; 95% CI, 2.7–3.4), CFS excess (adjusted OR, 4.8; 95% CI, 3.9–5.9), PTSD prevalence increased with combat stress intensity, from 3.3 to 22.6% (test for trend, $p > 0.15$)	Self-reported symptoms and exposures, lack of analysis solely of sarin-related exposure

continued

TABLE 3-2 Continued

Reference	Population	Exposure Assessment for Relevant Agents
Studies Reviewed in GW1		
Iowa Persian Gulf Study Group, 1997	1,896 deployed veterans from Iowa as home of record vs 1,799 nondeployed veterans from Iowa as home of record	One relevant exposure: "chemical warfare agents"
Goss Gilroy Inc., 1998	3,113 Canadian veterans deployed to Gulf War vs 3,439 deployed elsewhere	Over 30 exposures divided into six categories; one category was relevant: "chemical warfare agents" (nerve gas and mustard gas or other blistering agent)
Unwin et al., 1999	2,735 UK veterans deployed to Gulf War vs 2,393 deployed to Bosnia vs 2,422 deployed elsewhere	Three relevant environmental exposures: "NBC suits", "hear chemical alarms", "chemical/ nerve gas attack"

Timeframe and Health-Outcome Assessment	Results	Limitations
Symptom questionnaires, exposure questionnaire no more than 6 years after Khamisiyah demolition	In Gulf War veterans, exposure to "chemical warfare agents" associated with symptoms of cognitive dysfunction (prevalence difference, 6.8%; $p < 0.001$), depression (prevalence difference, 8.6%; $p < 0.001$), fibromyalgia (prevalence difference, 8.1%; $p < 0.001$)	Self-reported symptoms and exposures, low proportion of minority-group subjects, internal validation of responses not assessed, no adjustment for multiple comparisons, multiple associations between variety of exposures and variety of outcomes
Symptom questionnaires, exposure questionnaire 6 years after Khamisiyah demolition	In Gulf War cohort, exposure to "chemical warfare agents", in multivariate analysis, not associated with symptoms of cognitive dysfunction, chronic fatigue, fibromyalgia; significantly associated with PTSD diagnosed by health-care provider (OR, 5.25; 95% CI, 1.36–20.30) or symptom reporting (OR, 10.79; 95% CI, 3.11–37.49); "chemical warfare agents" also associated with symptoms of major depression (OR, 3.66; 95% CI, 1.21–11.03), anxiety (OR, 5.59; 95% CI, 1.48–21.07)	Self-reported symptoms and exposures, subset of Canadian veterans not exposed to many agents (because they were based at sea) reported symptoms as frequently as did land-based veterans, no adjustment for multiple comparisons, multiple associations between various exposures and various outcomes, not clear which relevant exposures related to outcome
Symptom questionnaires, exposure questionnaire, both 6–7 years after Khamisiyah demolition	In Gulf War cohort only, three exposures associated with chronic multisymptom illness and PTSD; for chronic multisymptom illness, ORs for three exposures, 2.2–2.7, CIs do not include 1; for PTSD, ORs for the three exposures, 2.1–3.1, CIs do not include 1	Self-reported symptoms and exposures, lack of adjustment for interrelationships between multiple exposures, use of p value of 0.05 despite multiple comparisons

continued

TABLE 3-2 Continued

Reference	Population	Exposure Assessment for Relevant Agents
Self-Reported Exposures: Military-Unit-Based Studies		
New Studies		
White et al., 2001	273 deployed veterans from Massachusetts (Fort Devens) and New Orleans vs 50 Germany-deployed veterans, 1994–1996 (same cohort as Proctor et al., 1998)	One relevant exposure: "chemical or biological warfare (CBW) agents"
Wolfe et al., 2002	1,290 Gulf War-deployed veterans from Massachusetts (Ft. Devens), 1997 (same cohort as Proctor et al., 1998)	Two relevant exposures: "exposure to poison gas or germ warfare" and "placement on formal alert for chemical and biological warfare"
Nisenbaum et al., 2000	1,002 US veterans from four Air Force units; nested case–control survey of 459 Gulf War veteran cases of chronic multisymptom illness vs 543 controls without chronic multisymptom illness (followup to Fukuda et al., 1998)	One relevant exposure: "thought biological or chemical weapons were being used"
Gray et al., 1999	527 active-duty US Seabees formerly deployed to Gulf War vs 969 nondeployed veterans from same Seabee commands	One relevant exposure: "chemical warfare"

Timeframe and Health-Outcome Assessment	Results	Limitations
15 neurobehavioral tests: WAIS-R, tests of attention, executive function, motor–psychomotor, visuospatial, memory, mood (POMS), motivation; exposure questionnaires; diagnostic interviews for PTSD; 3–5 years after Khamisiyah demolition	In regression analyses, Gulf War veterans exposed to CBW agents (vs nonexposed) more likely to have mood, memory, cognitive deficits; in particular, their scores significantly worse ($p < 0.05$) on POMS tension and confusion scales, three tests of recall memory, backward digit span test (WMS-R) of attention, executive system function (after controlling for PTSD and depression)	Self-reported exposures, not representative of entire Gulf War cohort
Psychologic-symptom questionnaire, combat-exposure questionnaire, 6 years after Khamisiyah demolition	In multivariate analysis, none of two exposures significantly associated with mild to moderate or severe multisymptom illness	Self-reported exposures, limited representativeness of entire Gulf War cohort
Symptom questionnaires, exposure questionnaire, 4 years after Khamisiyah demolition	"Thought biological or chemical weapons were being used", in logistic regression adjusting for presence of other exposures, associated with criteria for severe case of multisymptom illness (OR, 3.46; 95% CI, 1.73–6.91)and mild–moderate case (OR, 2.25; 95% CI, 1.54–3.27)	Self-reported symptoms and exposures, no reporting on exact time of exposure, exclusion of Gulf War veterans no longer in active service, no adjustment of p value despite multiple comparisons, limited representativeness of entire Gulf War cohort
Symptom questionnaire, exposure questionnaire, clinical examination, handgrip strength, pulmonary function, serum collection, covered from 1991–1995	"Chemical warfare" not 1 of the 11 exposures studied in analyses	Self-reported symptoms and exposures, potential recall bias in symptom reporting, moderate to low response rate, exclusion of veterans no longer in active service, results of multivariate analysis not reported, limited representativeness of entire Gulf War cohort

continued

TABLE 3-2 Continued

Reference	Population	Exposure Assessment for Relevant Agents
Gray et al., 2002	Gulf War-era active-duty and reserve US Seabees: 3,831 Gulf War Seabees, 4,933 Seabees deployed elsewhere, 3,104 nondeployed Seabees (followup to Gray et al., 1999)	One relevant exposure: "use of gas masks"
Kroenke et al., 1998	18,495 US Gulf War veterans in DOD Comprehensive Clinical Evaluation Program	One relevant exposure: "nerve gas/agents"
Studies Reviewed in GW1		
Proctor et al., 1998	291 deployed veterans from Massachusetts (Ft. Devens) and New Orleans vs 50 Germany-deployed veterans, 1994–1996	One relevant exposure: "chemical or biological warfare (CBW) agents"

Timeframe and Health-Outcome Assessment	Results	Limitations
Health behaviors; physician-diagnosed illnesses; self-reported persistent or recurring medical problems; exposure questionnaire, at least 6 years after Khamisiyah demolition	22% of Gulf War veterans met definition of Gulf War illness: 1 or more physician-diagnosed multisymptom illnesses or at least 12 self-reported persistent or recurring medical problems; in multivariate analysis, Gulf War illness associated with "use of gas masks" (OR, 1.40; 95% CI, 1.07–1.84)	Conducted 5–7 years after Gulf War, self-reported symptoms and exposures, potential recall bias in symptom reporting, limited representativeness of entire Gulf War cohort
Physician-administered symptom checklist, exposure questionnaire, combat and work-loss questionnaires no more than 6 years after Khamisiyah demolition	No association between individual symptoms and specific exposures	Included only subjects who presented for evaluation, self-reported symptoms and exposures, lack of control group, lack of statistical analysis, limited representativeness
Symptom questionnaires; exposure questionnaires; clinical evaluations for PTSD; evaluations conducted in 1991, 1993–1994, and 1995–1997	In Gulf War cohort, exposure to CBW agents, in multivariate analysis, significantly associated with musculoskeletal ($p = 0.001$),[a] neurologic symptoms ($p = 0.013$),[b] neuropsychologic ($p = 0.009$),[c] psychologic[d] ($p = 0.001$) symptoms	Self-reported symptoms and exposures, moderate to low response rate, limited representativeness of entire Gulf War cohort

continued

TABLE 3-2 Continued

Reference	Population	Exposure Assessment for Relevant Agents
Haley and Kurt, 1997	23 US veterans with up to three newly defined syndromes (derived from factor analysis) vs 229 veterans without newly defined syndromes	One relevant exposure: "chemical warfare agents"

[a]Joint pains, backaches, and neckaches or stiffness.
[b]Headaches, numbness in arms or legs, and dizziness.
[c]Difficulties in learning new material, difficulty in concentrating, and confusion.
[d]Inability to fall asleep, frequent periods of feeling depressed, and frequent periods of anxiety or nervousness.

have been exposed. That was based on proximity (within 50 km) to the demolition site. Subsequently, an exposure assessment was conducted that incorporated experimental tests for release rates for the chemicals, dispersion models that included weather estimates, and unit locations (CIA–DOD, 1997). Following criticisms of that exposure assessment, a revised exposure assessment was conducted that utilized updated unit locations, revised meteorologic models, and different estimates of sarin and cyclosarin releases (Winkenwerder, 2002). Although each subsequent exposure assessment technique improved upon the previous one, many uncertainties remain with respect to the actual exposures at Khamisiyah. No blood was tested for sarin exposures and, at this time, there is no way to determine what the actual exposures were. Despite those limitations, epidemiology studies have been conducted using the various exposure assessments that are available.

A number of studies published since the preparation of *GW1* (IOM, 2000) have looked at the health of soldiers potentially exposed to sarin at Khamisiyah. Gray et al. (1999) examined hospitalization over a 5-year timeframe (March 10, 1991–September 1995) in relation to potential exposure to nerve agents among 349,291 active-duty military deployed to the Gulf War theater during the time of the Khamisiyah demolition. Hospitalization experience was limited to active-duty military because they seldom receive care outside of Department of

Timeframe and Health-Outcome Assessment	Results	Limitations
Symptom questionnaire, exposure questionnaire, within 5 years of Gulf War	"Chemical warfare agents" exposure associated with one of three newly defined syndromes ("confusion–ataxia") (RR, 7.8; 95% CI, 2.3–25.9); synergy between exposure to "chemical warfare agents" and scores on scale of advance adverse effects from pyridostigmine bromide in predicting "confusion–ataxia syndrome"	Self-reported symptoms and exposures, no control group in original cohort, limited representativeness of entire Gulf War cohort

Abbreviations: CBW, chemical or biologic warfare; CFS, chronic fatigue syndrome; CI, confidence interval; DOD, Department of Defense; GI, gastrointestinal; MCS, multiple chemical sensitivity; NBC, nuclear, biologic, and chemical warfare; OR, odds ratio; POMS, profile of mood states; PTSD, posttraumatic stress disorder; RR, relative risk; UK, United Kingdom.

Defense (DOD) hospitals and because of the availability of automated data. Reserve veterans called to active duty were included only for the 3-month period of active-duty Gulf War service and potential Khamisiyah exposure (March 1991–June 10, 1991). The study included those on active duty after the end of the war and those retired with medical benefits. (When veterans return to reserve status, they are ineligible to receive care at DOD hospitals, but can be eligible for Veterans' Administration hospitals.) Exposure status was determined by whether active-duty military were within the plume area defined by meteorologic-dispersion modeling, according to DOD's initial plume-dispersion model (Rostker, 2000; Winkenwerder, 2002), and were within a military unit determined by geographic information systems data to have been exposed, during a 3-day period, to an extent set by the Centers for Disease Control and Prevention (CDC) as the general population limit (GPL), below which no symptoms were expected. Troops were considered exposed if concentrations were above the GPL of 0.0126 mg-min/m^3 for sarin and 0.00001 mg-min/m^3 for cyclosarin. Information on troop locations was provided from the Defense Manpower Data Center and Desert Shield-Desert Storm personnel files. Several exposure categories were created, but only three were used for studying possible dose–response relationships: not exposed (n = 224,804), uncertain low exposure (n = 75,717), and estimated subclinical exposure (n = 48,770). Three outcome measures were

used: hospitalizations for any cause, hospitalization diagnoses in 15 categories, and specific diagnoses proposed by an expert panel to be possible long-term effects of sarin, including such disorders as mononeuritis of the upper limb and myoneural disorders.

About 21% of active-duty personnel were hospitalized over the 5-year period regardless of exposure category. Cox proportional hazard modeling, adjusted for covariates, found that exposed and nonexposed personnel had similar adjusted risks for each category of hospitalization (all causes, 15 general categories, diagnosis-specific categories). The authors concluded that their data do not support a relationship between postwar hospitalizations and exposure to nerve-agent plume from demolition at Khamisiyah. The authors identified study limitations as restriction to DOD hospitals (because of the availability of computerized records), restriction to hospitalizations of active-duty Gulf War veterans who remained on active duty after the war, and restriction to hospitalization because outpatient data were unavailable. It was also not possible to adjust for potential confounding exposures.

Smith et al. (2003), in a followup of Gray et al. (1999), investigated hospitalizations over a 10-year period (March 10, 1991–December 31, 2000) among 418,072 (of 431,762) military personnel deployed to the Gulf War theater during the time of the Khamisiyah demolition on whom demographic and exposure data were complete and available. Of the personnel, 99,614 were considered exposed to sarin or cyclosarin, and 318,458 were considered nonexposed. The methods used were similar to those of Gray et al. (1999) except that exposure status was determined according to DOD's revised modeling in 2000 (Rostker, 2000; Winkenwerder, 2002) and the GPL was adjusted because of the briefer duration of troops' potential exposure. Troops were considered exposed at the exposure rate of 0.0432 mg-min/m^3 for sarin and 0.0144 mg-min/m^3 for cyclosarin. Outcome measures were the same as those of Gray et al. (1999).

Over the course of the 10-year observation time, no differences in the percentage of personnel hospitalized (18.4% of exposed, 18.8% of nonexposed) were seen. Exposed and nonexposed personnel also had similar attrition, about 57–58% separating from the military over the 10-year period. For any-cause hospitalization, the adjusted risk for exposed veterans was not significantly different from that for nonexposed veterans, but there were differences for some demographic and occupational variables. Using Cox's proportional hazard modeling, only one category of disease—circulatory system disease—showed a significant relationship with exposure (RR, 1.07; 95% CI, 1.0–1.13) after adjustment for other variables in the model. More specifically, only one of the 10 specific cardiac diagnoses was more frequent in the exposed than in the nonexposed: cardiac dysrhythmias (RR, 1.23; 95% CI, 1.04–1.44). The investigators, acknowledging that the finding could have resulted by chance, concluded that the excess in dysrhythmias was "small in comparison with potential

observational variability, but the findings are provocative and warrant further evaluation."

McCauley et al. (2001) published findings of self-reported symptoms based on questionnaire responses from a cohort of veterans deployed to the Gulf War from five states (Oregon, Washington, California, Georgia, and North Carolina). Those states were chosen because of the geographic distribution of units serving in the Khamisiyah area. The sample was divided into Gulf War veterans potentially exposed to sarin and cyclosarin on the basis of their units being located within 50 km (according to DOD information) of the demolition at Khamisiyah (Khamisiyah-exposed veterans; $n = 653$) and military personnel deployed to the Gulf War during the Gulf War combat period but not within 50 km of the Khamisiyah demolition (nonexposed veterans, $n = 610$). A third group, nondeployed veterans ($n = 516$), was drawn from the same states on the basis of their active duty or activation from reserve status during the Gulf War. The three groups were interviewed by telephone in 1999 about a 24-item list of self-reported symptoms during the 2-week period surrounding demolitions, their current symptoms, and more details about past exposure. In addition, McCauley et al. (2001) further divided the Khamisiyah-exposed veterans into those who reported—by responding yes to either "Involved in the munitions-demolition activity at Khamisiyah" or "Observed the demolition"—that they had been involved in or had watched the demolitions at Khamisiyah (Khamisiyah-witness, $n = 162$) and those who reported they had not been involved in and had not watched the demolitions at Khamisiyah (Khamisiyah-nonwitness, $n = 405$); the remainder of the 653 were unsure ($n = 86$).

There were no differences between the Khamisiyah-exposed and Khamisiyah-nonexposed groups in symptoms experienced during the first 2 weeks after the ground war. There were many differences, however, between the Khamisiyah-witness group and the Khamisiyah-nonwitness group. When questioned about symptoms that occurred at the time of demolitions, the Khamisiyah-witness group was more likely to recall 16 of 24 symptoms. They were also more likely to report three or more symptoms of sarin poisoning (vision problems, headache, running nose, coughing, tearing of eyes, and reddening of eyes) (OR, 2.13; 95% CI, 1.4–3.3). They were also, however, more likely to report three individual symptoms of mustard gas exposure (coarse voice, blisters on the skin, and rashes) despite the lack of evidence of such exposure and of a connection between those symptoms and sarin or cyclosarin exposure.

The veterans were also questioned about symptoms present in 1999, when the study was conducted. As in other Gulf War studies, both Gulf War-deployed groups (Khamisiyah-exposed and Khamisiyah-nonexposed) had higher current symptom reporting than did nondeployed veterans. However, the only difference between the two groups was that the Khamisiyah-exposed group reported fewer effects of being in a confined place (OR, 0.6; 95% CI, 0.4–0.9; OR adjusted for age, sex, and region of residence) than did the Khamisiyah-

nonexposed group. Significant differences (in ORs adjusted for age and sex) were seen in current symptom reporting between the Khamisiyah-witness group and the Khamisiyah-nonwitness group. The Khamisiyah-witness group reported more tingling or burning sensations of the skin (OR, 1.7; 95% CI, 1.1–2.8), changes in memory (OR, 1.7; 95% CI, 1.2–2.4), difficulty in sleeping (OR, 2.0; 95% CI, 1.2–3.5), persistent fatigue (OR, 1.8; 95% CI, 1.2–2.6), depression (OR, 1.6; 95% CI, 1.1–2.4), and bloody diarrhea (OR, 3.1; 95% CI, 1.6–6.0). In light of the excess of symptoms among those who self-reported witnessing the demolitions, the investigators concluded that the DOD 50-km designation was too broad. The investigators also pointed out that the current symptom excess among those witnessing the demolitions is consistent with chronic effects of low exposure to organophosphates.

The same research group conducted a factor analysis of the symptom findings from McCauley et al. (2001), in part to determine whether a unique pattern of symptoms was present among veterans potentially exposed to chemical-warfare agents (Shapiro et al., 2002). Although a number of symptoms were identified, no unique pattern of symptoms was identified in veterans who were potentially exposed to sarin and cyclosarin by being within 50 km of the Khamisiyah demolition (Khamisiyah-exposed). The investigators were unable to perform a factor analysis on the Khamisiyah-witness group, because there were too few in the group (n = 162).

McCauley et al. (2002) published a second study of veterans (n = 2,918) from the same five states as their earlier study (McCauley et al., 1999). The 2002 publication focused on self-reports, during a telephone interview, of physician-diagnosed health conditions, hospitalizations, and disability related to exposures at Khamisiyah. As in the earlier study, exposure was designated as being within 50 km of Khamisiyah. Khamisiyah-exposed Gulf War veterans were compared with Khamisiyah-nonexposed Gulf War veterans and nondeployed veterans. (No analyses of Khamisiyah-witness versus Khamisiyah-nonwitness groups were reported.) All health outcomes were based on veterans' self-reports regarding conditions diagnosed by a physician since the Gulf War, their employment status, hospitalizations, and disability. The method was the same as that of McCauley et al. (2001).

Deployed troops were significantly more likely than nondeployed troops to report physician-diagnosed high blood pressure (OR, 1.7; 95% CI, 1.3–2.4), heart disease (OR, 2.5; 95% CI, 1.1–6.6), slipped disk or pinched nerve (OR, 1.5; 95% CI, 1.1–2.0), PTSD (OR, 14.9; 95% CI, 5.6–60.9), hospitalization for depression (OR, 5.1; 95% CI, 1.5–32.1), and periodontal disease (OR, 1.8; 95% CI, 1.2–2.8). There was an excess of cancer in the deployed group, but the results became nonsignificant when skin cancer was removed from the analysis. None of the nearly 20 medical conditions, however, was reported to be more common among Khamisiyah-exposed than Khamisiyah-nonexposed Gulf War veterans. There

were no differences between deployed and nondeployed veterans in functional status, hospitalizations, and service-connected disability rates.

The committee is aware that currently a study is being conducted investigating the long-term health effects associated with potential low exposures to sarin and cyclosarin at Khamisiyah, Iraq, and the effect of notification of such exposure. That study, however, is not yet published.

Self-Reported Exposures

This section summarizes studies of Gulf War veterans in which possible indications of potential exposure to sarin or cyclosarin are self-reported. They include studies that report multiple symptoms and investigate their relationship to any response on a questionnaire or in an interview that might indicate potential exposure to an agent of interest to the present committee. This discussion focuses on the results that might be associated with potential exposures to sarin or cyclosarin. Most of the studies are large-scale epidemiologic studies of the health of veterans, including American, Canadian, British, and Danish veterans. A number of studies on those populations of veterans do not examine the relationship between symptoms and specific exposures or do not investigate exposures of interest to this committee, and such studies are not reviewed in this report. The studies summarized below are grouped according to whether the study design was population-based or military-unit-based. A population-based study is a methodologically robust type of epidemiologic study because its goal is to obtain information that is representative of the population of interest, in this case, Gulf War veterans. The cohort may be the entire population of interest or a random selection from the population of interest. Population-based studies of Gulf War veterans sample a cohort of veterans by contacting them where they live, as opposed to where they seek treatment or where they serve in the military (for example, a particular base, a particular branch such as the Air Force). Studies of military units or other military subgroups are less representative of the broader Gulf War veteran population than are population-based studies. The relevant findings are summarized in Table 3-2.

Population-Based Studies

The "Iowa study", a major population-based study of US Gulf War veterans reviewed in *GW1*, was a cross-sectional survey of a representative sample of 4,886 military personnel who had active military service at some time from August 2, 1990, to July 31, 1991, and who listed Iowa as their home of record at the time of enlistment (Iowa Persian Gulf Study Group, 1997). The study examined the health of military personnel in all branches of military service who were still serving or had left service. The sample was randomly selected from about

29,000 military personnel and was stratified by age, sex, rank, race, and type of military service. Of the eligible study subjects, 3,695 (76%) completed a telephone interview. Study subjects were divided into four groups: two that had been deployed to the Gulf War (n = 1,896) and two that had not (n = 1,799). Trained examiners using standardized questions, instruments, and scales interviewed the subjects by telephone.[1] The two groups deployed to the Gulf War reported roughly twice the prevalence of symptoms suggestive of the following conditions: fibromyalgia, cognitive dysfunction, depression, alcohol abuse, asthma, PTSD, sexual discomfort, or chronic fatigue.[2]

The Iowa study assessed exposure by asking Gulf War veterans to report on their exposures to over 20 items, one of which was "chemical-warfare agents". In Gulf War veterans, self-reported exposure to "chemical-warfare agents" was associated with symptoms of cognitive dysfunction (prevalence difference, 6.8% between exposed and nonexposed; $p < 0.001$), depression (prevalence difference, 8.6%; $p < 0.001$), and fibromyalgia (prevalence difference, 8.1%; $p < 0.001$). It was not reported whether there was a relationship between self-reported exposure to "chemical-warfare agents" and PTSD symptoms.

Goss Gilroy Inc. (1998; IOM, 2000) mailed a questionnaire to the entire cohort of almost 10,000 Canadian Gulf War veterans and Canadian forces deployed elsewhere during the same period. The response rate was 73% for Gulf War veterans and 60.3% for controls. Of the Gulf War veterans responding, 2,924 were male and 189 were female. Deployed forces (n = 3,113) had significantly higher rates than controls (n = 3,439) of self-reported chronic conditions and symptoms of a variety of symptom-derived clinical outcomes (chronic fatigue, cognitive dysfunction, multiple chemical sensitivities, major depression, PTSD, chronic dysphoria, anxiety, fibromyalgia, asthma, bronchitis, and respiratory diseases). Several of the reported health conditions or symptoms were combined to form clinically meaningful outcomes (Goss Gilroy Inc., 1998). The greatest differences between deployed and nondeployed forces were in chronic fatigue, cognitive dysfunction, and multiple chemical sensitivities.

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[1]Sources of questions included National Health Interview Survey, Behavioral Risk Factor Surveillance Survey, National Medical Expenditures Survey, Primary Care Evaluation of Mental Disorders, Brief Symptom Inventory, CAGE questionnaire, PTSD Checklist—Military, CDC Chronic Fatigue Syndrome Questionnaire, Chalder Fatigue Scale, American Thoracic Society questionnaire, Sickness Impact Profile, and questions to assess fibromyalgia, sexual functioning, and military exposures.

[2]Conditions listed were not diagnosed, because no clinical examinations were performed. Rather, before conducting their telephone survey, researchers grouped sets of symptoms from their symptom checklists into a priori categories of diseases or disorders. If a veteran identified himself or herself as having a requisite set of symptoms, researchers analyzing responses considered the veteran to have symptoms "suggestive" of or consistent with a particular disorder, but not its formal diagnosis.

In multivariate analyses, researchers examined over 30 self-reported exposures in six categories, two of which indicate a potential exposure to sarin or cyclosarin: self-reported exposure to "chemical-warfare agents", a category combining self-reported exposure to a nerve gas or to mustard gas or other blistering agent, and self-reported exposure to "psychologic stressors", which included answering yes to any of 10 occurrences, of which only two ("wearing protective gear other than for training" and "hearing chemical alarms sounding") might be indicative of potential exposure to sarin or cyclosarin. The heterogeneity of the "psychologic stressors" category, however, which included exposure to dead bodies of animals and humans, makes it difficult to tie any health effect directly to sarin-related exposures.

In the multivariate analysis, self-reported exposure to "chemical-warfare agents" was not associated with symptoms of cognitive dysfunction, chronic fatigue, or fibromyalgia. It was significantly associated with self-report of PTSD diagnosed by a health-care provider through a question on the symptom questionnaire rather than a structured clinical interview (OR, 5.25; 95% CI, 1.36–20.30) or by symptom reporting (OR, 10.79; CI, 3.11–37.49). Self-reported exposure to "chemical-warfare agents" was also associated with symptoms of major depression (OR, 3.66; CI, 1.21–11.03) and anxiety (OR, 5.59; CI, 1.48–21.07). A subset of Canadian veterans who were based at sea and could not have been exposed to many of the agents reported symptoms as frequently as did land-based veterans in this study. Also, no more than one-third of Canadian troops were on land in March, 1991, at the time of the Khamisiyah demolitions (personal communication with L. Smith; March 30, 2004).

Unwin et al. (1999; see IOM, 2000) investigated the health of servicemen from the UK in a cohort study. A survey was sent to 4,246 veterans—a stratified random sample of the entire UK contingent of about 53,000 personnel deployed to the Persian Gulf—and to two comparison groups. One of the comparison groups was deployed to the conflict in Bosnia ($n = 4,250$); this study was the only one to use a comparison population with combat experience during the time of the Gulf War. The second comparison group was deployed to other noncombat locations outside the UK over the same period ($n = 4,246$). The overall response rate was 65.1%. The study was stratified by branch of service, age, sex, regular or reserve status, rank, and fitness. Through a mailed questionnaire, the investigators asked about symptoms (50 items), medical disorders (39 items), and functional capacity. Many of the questions were taken from previously validated instruments, such as the Mississippi scale for PTSD, the SF-36, and the 12-item General Health Questionnaire. The principal outcome measures were the subjective health perception and physical functioning subscales of the SF-36. The authors used other item scales to put together syndromes to correspond to posttraumatic stress reaction or to the CDC-defined Gulf War syndrome. The study controlled for potential confounding factors (including sociodemographic and lifestyle factors) with logistic regression analysis. Only male veterans' results

were analyzed, because female veterans' roles and symptoms were distinct enough to warrant separate consideration.

Gulf War-deployed veterans reported higher prevalence of symptoms and diminished functioning than did both comparison groups. Gulf War veterans reported having the symptom-based criteria for chronic fatigue, posttraumatic stress reaction, and "chronic multisymptom illness" more often than comparison subjects. The fact that the Bosnia cohort, which also was deployed to a combat setting, reported fewer symptoms than the Gulf War cohort, suggests that combat deployment itself does not account for higher symptom reporting.

Of the more than 17 questions related to exposures, veterans' responses that they had used, heard, or been subjected to any of the following three situations might be relevant to potential sarin or cyclosarin exposure: "NBC suits" (NBC = nuclear, biologic, and chemical warfare), "hear chemical alarms", and "chemical/nerve gas attack". When the various exposures were analyzed, veterans reporting each of those three occurrences that might be relevant to sarin or cyclosarin had a higher likelihood of CDC multisymptom syndrome and posttraumatic stress reaction. The odds of having chronic CDC multisymptom illness were higher in veterans who reported any of those three occurrences than in those who did not report them (OR, 2.7; 95% CI, 2.3–3.3; OR, 2.2; 95% CI, 1.9–2.6 and OR 2.6; 95% CI, 1.9–3.5 for responding yes to the three items, respectively), as were the odds of having posttraumatic stress (OR, 3.0; 95% CI, 2.1–4.4; OR 2.1, 95% CI, 1.6–2.8; and OR 3.1, 2.3–4.1 for responding yes to the three items, respectively). Those analyses, however, did not include a correction for multiple comparisons, and some of the ORs for the same self-reported exposures and health outcomes were also increased in Bosnia-deployed veterans and era veterans, for both of whom there is no evidence of possible sarin or cyclosarin exposures; suggesting that perception of the event, rather than sarin or cyclosarin itself, might be causing the effect. The authors interpreted the findings as evidence that the exposures were not specifically associated with Gulf War-related illnesses.

Since the preparation of *GW1* (IOM, 2000), Reid et al. (2001) further analyzed the results from Unwin et al. (1999) to estimate the prevalence of multiple chemical sensitivity (MCS) and chronic fatigue syndrome (CFS) and to determine whether these two conditions were associated with the same self-reported exposures or occurrences discussed above. The MCS case definition required symptoms in at least three organ systems (including the CNS) for 3 months or more and self-reported sensitivity to four or more substances on a list of 11; this combination was chosen to correspond as closely as possible to the criteria established by Simon et al. (1993). A case of CFS was defined by using veterans' responses to the study's fatigue scale and the SF-36 measure of functional disability; this combination was chosen to approximate the CDC criteria for CFS (Fukuda et al., 1994). Exposures and the response rate were the same as for Unwin et al. (1999). The prevalence of MCS was significantly greater in Gulf War veterans (1.3%) than in the two control groups (adjusted OR, 7.2; 95% CI,

2.8–18.2). In Gulf War veterans, MCS was associated with self-reports of hearing chemical alarms (OR, 2.5; 95% CI, 1.0–5.9) and self-reports of having a chemical or nerve-gas attack (OR, 4.6; 95% CI 1.6–13.3). The prevalence of CFS in Gulf War veterans (2.1%) was not significantly higher than that in one of the two control groups. It was, however, significantly associated with self-reports of hearing chemical alarms (OR, 2.5; 95% CI, 1.2–5.3).

Danish Persian Gulf veterans were almost all involved in peacekeeping or humanitarian roles after the end of the war, from the end of April 1991 through 1996 (Ishoy et al., 1999b). The vast majority of those veterans, therefore, were not in the Persian Gulf at the time of the Khamisiyah demolitions. A number of studies have been conducted on Danish gulf veterans, including studies of symptoms and diseases (Ishoy et al., 1999a), male reproductive problems (Ishoy et al., 2001a) and sexual problems (Ishoy et al., 2001b), gastrointestinal problems (Ishoy et al., 1999b), and neuropsychologic symptoms (Suadicani et al., 1999). Only the latter two studies, however, examined possible relationships between potential chemical exposures and health outcomes. Danish troops were successively replaced every 6 months, so most respondents were not in the gulf until years after the end of the war; about 60% were deployed between 1992 and 1994, and 20% after 1995. The gulf-area veterans were matched by age, sex, and profession to 400 members of the Danish armed forces who could have been, but were not, deployed to the gulf.

Suadicani et al. (1999) investigated whether 22 self-reported neuropsychologic symptoms in Danish gulf veterans were associated with self-reported exposures assessed up to 6 years after the war. A total of 821 veterans were eligible for the study by virtue of having served at any time during August 1990–December 1997. The response rate was 84% and 58% in gulf-area veterans and controls, respectively (Ishoy et al., 1999b). A combination of health and exposure questionnaires and health examinations were used, but only the results of the questionnaires are reported in Suadicani et al. (1999). The symptom questionnaire contained 17 neuropsychologic symptoms. Each of the 17 self-reported symptoms was significantly more prevalent among gulf-area veterans than controls; many of the symptoms were correlated with one another. Multiple logistic regression analysis with adjustments for age and sex was used to find the "most relevant" symptoms. Five of the 17 symptoms (concentration or memory problems, repeated fits of headache, balance disturbances or fits of dizziness, abnormal fatigue not caused by physical activity, and problems in sleeping all night) remained significant after this analysis. About 21% of gulf-area veterans reported a clustering of three to five of these relevant symptoms versus 6.2% of controls ($p < 0.001$). Of the 26 questions regarding exposures that are reported by Suadicani et al. (1999; see Table 4 and its footnote), only self-reported exposure to "nerve gas" is potentially relevant to the present report. Self-reported exposure to "nerve gas" was not significantly associated with the neuropsychologic symptoms in the gulf-area veterans.

Ishoy et al. (1999b) studied gastrointestinal (GI) symptoms in Danish gulf-area veterans and matched controls. On a questionnaire, eight of 14 GI symptoms were reported significantly more frequently in veterans than in controls. After adjustment for the interrelationship of variables, only two of the eight GI symptoms remained significant: 1-year prevalence of recurrent diarrhea and rumbling in the stomach more than twice a week. Ishoy et al. (1999b) used both symptoms as the combined main GI outcome measure and investigated its relationship to numerous environmental exposures. In the gulf-area cohort, of 24 exposures reported in the article, only self-reported exposure to "nerve gas" is relevant to the present report, and it was not significantly associated with the main GI outcome measure in univariate and multivariate analyses.

Spencer et al. (2001) conducted a nested case–control study of the exposure-symptom relationships in Gulf War veterans with unexplained illness (n = 241) and healthy Gulf War-deployed controls (n = 113) drawn from their population-based sample of 23,711 Gulf War veterans from Oregon or Washington state. Clinical evaluation was conducted to verify symptoms and to exclude known conditions that could explain them. The self-reported exposure questionnaire was reduced from 144 items to 44 items on the basis of test–retest reliability and other factors. Of the 44 exposures asked about, three are potentially relevant to the present report: "chemical decontamination bottles", "inadequate protection during chemical/SCUD alarms", and "worked around chemical-warfare agents". Only answering yes to "inadequate protection during chemical/SCUD alarms" was found (with simple logistic regression) to be associated with unexplained illness defined by this research team (see McCauley et al., 1999 for description; OR, 2.39; 95% CI, 1.03–5.56) and by CDC's case definition of multisymptom illness (OR, 3.16; 95% CI, 1.28–7.80). None of the three self-reported exposures that are potentially relevant to the present report was assessed in the multivariate analysis.

The cases of unexplained illness defined in Spencer et al. (2001) have been followed up with neurobehavioral and other testing. Storzbach et al. (2000) found that subjects (n = 241) had small but statistically significant deficits on some neurobehavioral tests of memory, attention, and response speed and were significantly more likely to report increased distress and psychiatric symptoms than controls (n = 113). A later analysis focused on a subgroup of 30 of the 241 whose performance was slowest on the Oregon Dual Task Procedure (ODTP), a relatively new test of digit recognition that assesses motivation, attention, and memory (Storzbach et al., 2001). In comparison with other subjects, the "slow ODTP" group performed worse on other neurobehavioral tests of memory, attention, and reaction time but not on psychologic tests. None of those studies, however, examined symptoms in relation to any potential exposures in the Gulf War.

Kang et al. (2000) conducted a large population-based cohort study of Gulf War veterans. Although they did not assess the relationship between any potential exposures and symptoms in that study (11,441 Gulf War veterans and 9,476

non-Gulf War veterans who were in the military at the time of the war but were not deployed to the Gulf War), they have since investigated potential relationships between symptoms and possible exposures in two nested case–control studies (Kang et al., 2002, 2003).

Kang et al. (2002) conducted factor analyses of 47 symptoms that were included in the earlier questionnaire (Kang et al., 2000) responded to by the cohort of Gulf War veterans (n = 10,423) and non-Gulf War veterans controls (n = 8,960) (participants with incomplete survey data were excluded from the analyses). The factor analyses were conducted in an attempt to identify one or more clusters of symptoms in the two groups. On the basis of the analyses, it was possible that a cluster of four symptoms—loss of balance or dizziness, speech difficulty, blurred vision, and tremors or shaking—made up a possible syndrome unique to veterans deployed to the Gulf. A group of 277 of the deployed veteran respondents (2.4%) and 43 of the non–Gulf War-deployed veterans (0.45%) met the case definition, reporting all four symptoms. The results of the analyses were then examined to determine which of 23 possible exposures based on self-reports were more common among the 277 Gulf War-deployed veterans who exhibited all four symptoms (cases) than among Gulf War veterans who lacked any of the four symptoms (controls; n = 6,730). Cases reported exposure to nine potential exposures on the questionnaire at a rate three or more times higher than Gulf War veterans who did not exhibit all four symptoms (controls). Self-reported exposure to "nerve gas" is the only one of those potential exposures relevant to the present report. Exposure to "nerve gas" was reported by 42.3% of deployed cases and 4.6% of deployed controls.

In another nested case–control study, Kang et al. (2003) evaluated the prevalence of PTSD and CFS in the same cohort of Gulf War and non-Gulf War veterans discussed above and investigated whether the "extent of deployment-related stress" was related to either syndrome. Although "wore chemical protective gear (other than for training) or heard chemical alarms sounding" was one of three experiences[3] that were used to define the high-stress group, the analyses were not reported with respect to the number of stressors present (0, 1, 2, or 3) and no data specific to "wore chemical protective gear (other than for training) or heard chemical alarms sounding" were presented. The study did find that an increased number of stressors was related, in a dose-related manner, to an increased likelihood of PTSD.

Military-Unit-Based Studies

A number of studies of veterans potentially exposed to sarin at Khamisiyah were discussed in *GW1*. In a series of studies of members of a naval battalion

[3] The other two were "involved in direct combat duty" and "witnessed any deaths".

called to active duty for the Gulf War, Haley and Kurt (1997) found that veterans who believed themselves to have been exposed to chemical weapons[4] were more likely to be classified as having "confusion–ataxia", one of six new proposed syndromes, which features problems with thinking, disorientation, balance disturbances, vertigo, and impotence (Haley et al., 1997a). A follow-up study of vestibular function was performed on a subset of veterans who had the highest factor scores on three of the syndromes identified in 1997 by Haley and Kurt (Roland et al., 2000). The study was designed to probe the nature of veterans' vestibular symptoms, rather than to examine the relationship between vestibular performance and exposure in the Gulf War. The study concluded that there was subjective and objective evidence of injury to the vestibular system in this group of Gulf War veterans with newly defined syndromes. Haley and Kurt (1997) hypothesized that those chronic syndromes represent variants of OPIDN.

Haley et al. (1997a; see IOM, 2000) conducted factor analysis to define a potential cluster of veterans' unexplained symptoms to define a unique syndrome. The analysis was conducted on data collected from reservists of the Naval Mobile Construction Battalion 24 (Seabees) who were called to active duty for the Gulf War and had worked in Saudi Arabia building airports, ammunition supply points, and roads. Of the 606 battalion members, 249 (41%) responded to the survey. Of the 249, 175 (79%) reported having had a serious health problem since returning from the Gulf War, and the other 74 (30%) reported having no serious health problems. A list of 22 major symptoms was developed from the major symptoms commonly associated with Gulf War illness in the clinical examinations performed by DOD and Veterans Administration (VA) physicians. Survey booklets were prepared that required a yes or no response for the presence of each of those symptoms. When veterans responded yes to having a major symptom, they were presented with a battery of 4 to 20 follow-up questions designed to define their exact symptoms (for example, to differentiate daytime sleepiness from muscle exhaustion after exercise when veterans reported "fatigue"). A factor analysis was done for each of the 22 major symptoms with the responses to the followup items. The result of that analysis was 51 "unambiguous symptom factors that appeared clinically meaningful". Those 51 factors were converted to symptom scales, to which was added a 52nd scale to measure tinnitus. A second factor analysis was then conducted with the 52 symptom scales to attempt to cluster symptoms and define one or more Gulf War syndromes. Through that process, six syndromes were defined: impaired cognition, confusion–ataxia, arthromyoneuropathy, phobia–apraxia, fever–adenopathy, and weakness–incontinence. The distribution of the 249 veterans

[4]On the basis of self-reports about their perceptions of chemical-warfare exposure rather than evidence of symptoms. Their location in relation to the Khamisiyah demolition site was not reported. The questionnaire was sent to participants in 1994, before the Department of Defense reported that chemical-weapons exposure could have occurred.

on each of the six syndrome factor scales was plotted in a single scatter plot. Visual inspection of the distributions suggested that a factor score of 1.5 would be appropriate for dichotomizing each of the six factor scales. Any veteran scoring higher than 1.5 on a given factor scale was said to have a case of that "syndrome". About one-fourth of the veterans (63) were classified as having one or more of the six syndromes. The first three syndromes had the strongest clustering of symptoms.

Haley and Kurt (1997; see IOM, 2000) investigated the relationship between those symptom clusters and self-reported exposure to organophosphates and related chemicals that inhibit cholinesterase. Specifically, the relationship between the three "syndromes" identified as having the strongest clustering—(1) impaired cognition, (2) confusion–ataxia, and (3) arthromyoneuropathy (Haley et al., 1997a)—was examined. Exposures of veterans with syndrome 1 (12 veterans), 2 (21 veterans), or 3 (22 veterans) were compared with veterans in the same battalion who did not have the syndrome (controls). Some individuals had more than one syndrome. At the time of the initial data collection, investigators had asked 249 participants about their exposures via a detailed questionnaire on 18 potential exposures during the Gulf War. Those reporting an exposure were asked additional questions to elicit duration and dose of exposure, anatomic areas exposed, and other modifying information. One question, whether the veteran was exposed to "chemical-warfare agents", is relevant to the present report. Each of the three syndromes was first screened against all the risk-factor variables in a univariate analysis. Adjusted step-wise logistic regressions were then performed with each syndrome to adjust for correlations between exposures. The p criterion was set at 0.005 because of the multiple comparisons performed. Exposure to "chemical-warfare agents" was associated with one of three newly defined syndromes, "confusion–ataxia" (RR, 7.8; 95% CI, 2.3–25.9). That particular syndrome featured problems with thinking, disorientation, balance, vertigo, and impotence. The investigators also found synergy between exposure to "chemical-warfare agents" and scores on a scale of adverse effects of pyridostigmine bromide for the confusion–ataxia syndrome.

At least four additional studies by Haley and collaborators delineate further the nature of the neurotoxic deficit in veterans (n = 20–23) with syndrome 1, syndrome 2, or syndrome 3 (Haley et al., 1997b, 1999, 2000; Hom et al., 1997; Roland et al., 2000). None of those studies, however, examined the relationship between the presence of the syndromes and possible exposures during service in the Persian Gulf.

Gray et al. (1999) assessed symptom–exposure relationships in Seabees but limited their cohort to active-duty Seabees (that is, they did not include reservists) who remained in the Navy at the time of the study. Both Gulf War-deployed Seabees and Seabees not deployed to the Gulf War were evaluated. The exposure questionnaire for the Seabees included a question about exposure to "chemical-warfare". Self-reported exposure to "chemical-warfare" was greater in the Gulf

War veterans than in the nondeployed veterans (OR, 3.1; 95% CI, 1.5–6.3; prevalence in Gulf War veterans, 3.2%). Further analysis of the symptom–exposure relationships, however, was limited to exposures with a prevalence of at least 5% or an OR of at least 3.0 to reduce the number of exploratory comparisons. Therefore, associations between symptoms and "chemical-warfare" exposure were not analyzed.

Beginning in May 1997, Gray et al. (2002) conducted a larger investigation of all Gulf-War era Seabees, regardless of whether they were still in active service. Of the 18,945 eligible Seabees, 11,868 (63%) completed the questionnaire (3,831 Gulf War Seabees, 4,933 Seabees deployed elsewhere, and 3,104 nondeployed Seabees). Survey questionnaires covered health behaviors, self-reported physician-diagnosed illnesses, and self-reported persistent or recurring medical problems. Gulf War-deployed Seabees were also asked about 34 possible exposures in the Gulf War. Gulf War Seabees were more likely to be smokers or to have been smokers in the past, to report being in fair or poor health, to report all 33 self-reported persistent or recurring medical problems, and to report a variety of physician-diagnosed illnesses, particularly CFS, PTSD, MCS, and irritable bowel syndrome. There was a high correlation between those four multisymptom illnesses, high scores on the Cognitive Failures Questionnaire, and self-reporting of 12 or more medical problems. The investigators created a case definition of Gulf War illness: having one or more of the four self-reported physician-diagnosed multisymptom illnesses or at least 12 self-reported persistent or recurring medical problems. Of the Gulf War veterans, 22% met the case definition. The authors report that "no Seabees had been located under the atmospheric plume subsequent to the March 1991 destruction of munitions at the Khamisiyah site", a statement for which they cited another of their studies (Gray et al., 1999). Using multivariate logistic regression, they found that 12 of 34 Gulf War-related exposures were associated with the case definition. One was relevant to the present report: "use of gas masks" (OR, 1.40; 95% CI, 1.07–1.84).

Nisenbaum et al. (2000) used a nested case–control design to determine whether environmental exposures were associated with cases of chronic multisymptom illness versus Gulf War-deployed controls without the illness. Overall, 1,002 of 1,155 originally contacted veterans were surveyed, for a response rate of 87%. The 459 veterans who met case criteria were divided into "severe" and "mild–moderate" cases according to symptom severity. Of the six environmental exposures that were studied in the exposure section of the questionnaire, one was relevant to the present report: "thought biological or chemical weapons were being used". Through logistic regression that adjusted for the presence of other exposures, that exposure was associated with meeting criteria for a severe case of multisymptom illness (OR, 3.46; 95% CI, 1.73–6.91) and a mild–moderate case (OR, 2.25; CI, 1.54–3.27).

The symptom experience of Gulf War veterans from Massachusetts (Ft. Devens) and New Orleans was studied by Proctor and colleagues in a series of

longitudinal studies. The original cohort included 2,949 troops from Ft. Devens and 928 from New Orleans; both groups consisted of active-duty, reserve, and National Guard troops deployed to the gulf. Studies were carried out in 1991, 1992–1993, and 1994–1996. The 1994–1996 study (Proctor et al., 1998) was the first to examine symptom–exposure relationships. The study's nearly 300 subjects represented a stratified random sample of troops who had participated in an earlier survey, selected to give equal representation of higher and lower symptom reporters. The participation rates were 58% (Ft. Devens) and 85% (New Orleans) of those who participated in the earlier study and who could be located and contacted. The control group was 50 Gulf War-era veterans deployed to Germany (85% participation rate). Subjects were given symptom checklists (covering the previous 30 days), exposure questionnaires, and a neuropsychologic test battery and were interviewed about combat exposure and PTSD. Each of the 52 symptoms on the symptom checklist was assigned to one of nine body systems by four independent judges (an occupational-health physician, an environmental-health specialist, an environmental epidemiologist, and a neuropsychologist). A score was calculated for each body system, on the basis of the number of symptoms reported. The exposure questionnaire, given only to Gulf War-deployed subjects, contained eight items, one of which, self-reported "exposure to chemical or biological warfare (CBW) agents", was relevant to the present report.

Relationships between self-reported exposures and body-symptom scores were examined in the Gulf War-deployed cohort, and analyses were restricted to exposure–symptom pairs for which there was a supporting a priori hypothesis. In multiple regression—adjusting for age, sex, education, war-zone stressors, and PTSD diagnosis—self-reported exposure to CBW agents was significantly associated with musculoskeletal[5] ($p = 0.001$), neurologic[6] ($p = 0.013$), neuropsychologic[7] ($p = 0.009$), and psychologic[8] ($p = 0.001$) symptoms but not dermatologic symptoms, whether the exposure was evaluated individually or simultaneously with all other exposure variables in the regression model. The findings were unchanged when subjects who met criteria for PTSD were removed from analyses.

White et al. (2001) also studied the cohort of Gulf War veterans from Massachusetts (Ft. Devens) and New Orleans. A total of 343 subjects participated in at least one part of the study: 293 Gulf War-deployed veterans and 50 Gulf War-era veterans deployed to Germany. Subjects were given a battery of neuropsychologic tests and an exposure questionnaire. The neuropsychological test battery covered

[5]Joint pains, backaches, and neckaches or stiffness.

[6] Headaches, numbness in arms or legs, and dizziness.

[7] Difficulties in learning new material, difficulty concentrating, and confusion.

[8] Inability to fall asleep, frequent periods of feeling depressed, and frequent periods of anxiety or nervousness.

52 measures involving seven functional domains: general intelligence, attention and executive function, motor ability, visuospatial processing, verbal and visual memory, mood, and motivation. The exposure questionnaire asked about eight environmental exposures, one of which, "exposure to chemical or biological warfare (CBW) agents", was relevant to the present report. In regression analyses, Gulf War veterans exposed to CBW agents were more likely than nonexposed Gulf War veterans to have mood, memory, and cognitive deficits. In particular, their scores were significantly worse ($p < 0.05$) on profile of mood states, tension and confusion scales, and tests of recall memory (including the backward digit span test, WMS-R). The authors attempted to control for PTSD, depression, and other covariates in the analyses, however, the ability to control for those covariates in this situation is questionable.

In a series of longitudinal studies of a cohort of Gulf War veterans from Massachusetts (Ft. Devens) in 1997, 1,290 subjects were given a 52-item health questionnaire of symptoms and a separate questionnaire about 12 environmental exposures (Wolfe et al., 2002). Two of the exposures were relevant to the present report: "exposure to poison gas or germ warfare" and "placement on formal alert for chemical and biological warfare". About 60% of respondents met CDC's case criteria for multisymptom illness (either a "mild-to-moderate" or "severe" case) (Fukuda et al., 1998). In Gulf War-deployed veterans, univariate analyses revealed an association between high frequency of "placement on formal alert for chemical and biological warfare" and mild-to-moderate or severe multisymptom illness. After multivariate adjustment, neither of the two exposures was associated with multisymptom illness.

Kroenke et al. (1998) reported on a case series of 18,495 Gulf War veterans who sought a medical evaluation through the DOD volunteer registry program known as the Comprehensive Clinical Evaluation Program. The evaluation followed a standard protocol that included a structured clinical assessment, a physician-administered symptom checklist, and a questionnaire about self-reported exposures, combat experiences, and work loss. One of 18 exposures, "nerve gas/agents", was relevant to the present report. The authors indicate that "no apparent association" between individual symptoms and any of the specific exposures was found, and that no tests of statistical significance were conducted, citing the large sample and multiple comparisons.

REFERENCES

Asukai N, Maekawa K. 2002. Psychological and physical health effects of the 1995 Sarin attack in the Tokyo subway system. In: Havenaar J, Cwikel J, eds. *Toxic Turmoil: Psychological and Societal Consequences of Ecological Disasters.* New York, NY: Kluwer Academic/Plenum Publishers. Pp. 149–162.

Asukai N, Kato H, Kawamura N, Kim Y, Yamamoto K, Kishimoto J, Miyake Y, Nishizono-Maher A. 2002. Reliability and validity of the Japanese-language version of the impact of event scale-revised (IES-R-J): Four studies of different traumatic events. *Journal of Nervous Mental Diseases* 190(3):175–182.

Baker DJ, Sedgwick EM. 1996. Single fibre electromyographic changes in man after organophosphate exposure. *Human and Experimental Toxicology* 15(5):369–375.

Black RM, Harrison JM, Read RW. 1999. The interaction of sarin and soman with plasma proteins: The identification of a novel phosphonylation site. *Archives of Toxicology* 73(2):123–126.

Brown MA, Brix KA. 1998. Review of health consequences from high-, intermediate- and low-level exposure to organophosphorous nerve agents. *Journal of Applied Toxicology* 18(6):393–408.

Burchfiel JL, Duffy FH. 1982. Organophosphate neurotoxicity: Chronic effects of sarin on the electroencephalogram of monkey and man. *Neurobehavioral Toxicology and Teratology* 4(6):767–778.

Cherry N, Creed F, Silman A, Dunn G, Baxter D, Smedley J, Taylor S, Macfarlane GJ. 2001. Health and exposures of United Kingdom Gulf War veterans. Part I: The pattern and extent of ill health. *Occupational and Environmental Medicine* 58(5):291–298.

CIA–DOD (Central Intelligence Agency and Department of Defense). 1997. *Modeling the Chemical Warfare Agent Release at the Khamisiyah Pit*. Washington, DC: CIA–DOD.

Duffy FH, Burchfiel JL, Bartels PH, Gaon M, Sim VM. 1979. Long-term effects of an organophosphate upon the human electroencephalogram. *Toxicology and Applied Pharmacology* 47(1):161–176.

Fukuda K, Straus SE, Hickie I, Sharpe MC, Dobbins JG, Komaroff A. 1994. The chronic fatigue syndrome: A comprehensive approach to its definition and study. International Chronic Fatigue Syndrome Study Group. *Annals of Internal Medicine* 121(12):953–959.

Fukuda K, Nisenbaum R, Stewart G, Thompson WW, Robin L, Washko RM, Noah DL, Barrett DH, Randall B, Herwaldt BL, Mawle AC, Reeves WC. 1998. Chronic multisymptom illness affecting Air Force veterans of the Gulf War. *Journal of the American Medical Association* 280(11):981–988.

Goss Gilroy Inc. 1998. *Health Study of Canadian Forces Personnel Involved in the 1991 Conflict in the Persian Gulf,* Vol. 1. Ottawa, Ontario: Goss Gilroy Inc. Prepared for the Department of National Defence.

Gray GC, Kaiser KS, Hawksworth AW, Hall FW, Barrett-Connor E. 1999. Increased postwar symptoms and psychological morbidity among US Navy Gulf War veterans. *American Journal of Tropical Medicine and Hygiene* 60(5):758–766.

Gray GC, Reed RJ, Kaiser KS, Smith TC, Gastanaga VM. 2002. Self-reported symptoms and medical conditions among 11,868 Gulf War-era veterans: The Seabee Health Study. *American Journal of Epidemiology* 155(11):1033–1044.

Haley RW, Kurt TL. 1997. Self-reported exposure to neurotoxic chemical combinations in the Gulf War. A cross-sectional epidemiologic study. *Journal of the American Medical Association* 277(3):231–237.

Haley RW, Kurt TL, Hom J. 1997a. Is there a Gulf War syndrome? Searching for syndromes by factor analysis of symptoms. *Journal of the American Medical Association* 277(3):215–222.

Haley RW, Hom J, Roland PS, Bryan WW, Van Ness PC, Bonte FJ, Devous MDS, Mathews D, Fleckenstein JL, Wians FH Jr, Wolfe GI, Kurt TL. 1997b. Evaluation of neurologic function in Gulf War veterans. A blinded case–control study. *Journal of the American Medical Association* 277(3):223–230.

Haley RW, Billecke S, La Du BN. 1999. Association of low PON1 type Q (type A) arylesterase activity with neurologic symptom complexes in Gulf War veterans. *Toxicology and Applied Pharmacology* 157(3):227–233.

Haley RW, Fleckenstein JL, Marshall WW, McDonald GG, Kramer GL, Petty F. 2000. Effect of basal ganglia injury on central dopamine activity in Gulf War syndrome: Correlation of proton magnetic resonance spectroscopy and plasma homovanillic acid levels. *Archives of Neurology* 57(9):1280–1285.

Hom J, Haley RW, Kurt TL. 1997. Neuropsychological correlates of Gulf War syndrome. *Archives of Clinical Neuropsychology* 12(6):531–544.

IOM (Institute of Medicine). 2000. *Gulf War and Health, Volume 1: Depleted Uranium, Pyridostigmine Bromide, Sarin, and Vaccines*. Washington, DC: National Academy Press.

IOM. 2003. *Gulf War and Health, Voume 2: Insecticides and Solvents*. Washington, DC: The National Academies Press.

Iowa Persian Gulf Study Group. 1997. Self-reported illness and health status among Gulf War veterans: A population-based study. *Journal of the American Medical Association* 277(3):238–245.

Ishoy T, Suadicani P, Guldager B, Appleyard M, Gyntelberg F. 1999a. Risk factors for gastrointestinal symptoms. *Danish Medical Bulletin* 46(5):420–423.

Ishoy T, Suadicani P, Guldager B, Appleyard M, Hein HO, Gyntelberg F. 1999b. State of health after deployment in the Persian Gulf. *Danish Medical Bulletin* 46(5):416–419.

Ishoy T, Andersson AM, Suadicani P, Guldager B, Appleyard M, Gyntelberg F, Skakkebaek NE. 2001a. Major reproductive health characteristics in male Gulf War Veterans. The Danish Gulf War Study. *Danish Medical Bulletin* 48(1):29–32.

Ishoy T, Suadicani P, Andersson A-M, Guldager B, Appleyard M, Skakkebaek NE, Gyntelberg F. 2001b. Prevalence of male sexual problems in the Danish Gulf War study. *Scandinavian Journal of Sexology* 4(1):43–55.

Kang HK, Mahan CM, Lee KY, Magee CA, Murphy FM. 2000. Illnesses among United States veterans of the Gulf war: A population-based survey of 30,000 veterans. *Journal of Occupational and Environmental Medicine* 42(5):491–501.

Kang HK, Mahan CM, Lee KY, Murphy FM, Simmens SJ, Young HA, Levine PH. 2002. Evidence for a deployment-related Gulf War syndrome by factor analysis. *Archives of Environmental Health* 57(1):61–68.

Kang HK, Natelson BH, Mahan CM, Lee KY, Murphy FM. 2003. Post-traumatic stress disorder and chronic fatigue syndrome-like illness among Gulf War veterans: A population-based survey of 30,000 veterans. *American Journal of Epidemiology* 157(2):141–148.

Kawana N, Ishimatsu S, Kanda K. 2001. Psycho-physiological effects of the terrorist sarin attack on the Tokyo subway system. *Military Medicine* 166(12 Suppl):23–26.

Kroenke K, Koslowe P, Roy M. (Regenstrief Institute for Health Care, Indianapolis, IN 46202-2859, USA). 1998. Symptoms in 18,495 Persian Gulf War veterans. Latency of onset and lack of association with self-reported exposures [comment]. *Journal of Occupational and Environmental Medicine* 40(6):520–528.

McCauley LA, Joos SK, Lasarev MR, Storzbach D, Bourdette DN. 1999. Gulf War unexplained illnesses: Persistence and unexplained nature of self-reported symptoms. *Environmental Research* 81(3):215–223.

McCauley LA, Rischitelli G, Lambert WE, Lasarev M, Sticker DL, Spencer PS. 2001. Symptoms of Gulf War veterans possibly exposed to organophosphate chemical warfare agents at Khamisiyah, Iraq. *International Journal of Occupational and Environmental Health* 7(2): 79–89.

McCauley LA, Lasarev M, Sticker D, Rischitelli DG, Spencer PS. 2002. Illness experience of Gulf War veterans possibly exposed to chemical warfare agents. *American Journal of Preventive Medicine* 23(3):200–206.

Murata K, Araki S, Yokoyama K, Okumura T, Ishimatsu S, Takasu N, White RF. 1997. Asymptomatic sequelae to acute sarin poisoning in the central and autonomic nervous system 6 months after the Tokyo subway attack. *Journal of Neurology* 244(10):601–606.

Nakajima T, Sato S, Morita H, Yanagisawa N. 1997. Sarin poisoning of a rescue team in the Matsumoto sarin incident in Japan. *Occupational and Environmental Medicine* 54(10):697–701.

Nakajima T, Ohta S, Morita H, Midorikawa Y, Mimura S, Yanagisawa N. 1998. Epidemiological study of sarin poisoning in Matsumoto City, Japan. *Journal of Epidemiology* 8(1):33–41.

Nakajima T, Ohta S, Fukushima Y, Yanagisawa N. 1999. Sequelae of sarin toxicity at one and three years after exposure in Matsumoto, Japan. *Journal of Epidemiology* 9(5):337–343.

Nisenbaum R, Barrett DH, Reyes M, Reeves WC. 2000. Deployment stressors and a chronic multisymptom illness among Gulf War veterans. *Journal of Nervous and Mental Disease* 188(5):259–266.

Nishiwaki Y, Maekawa K, Ogawa Y, Asukai N, Minami M, Omae K. 2001. Sarin Health Effects Study Group. Effects of sarin on the nervous system in rescue team staff members and police officers 3 years after the Tokyo subway sarin attack. *Environmental Health Perspectives* 109(11):1169–1173.

Nohara M. 1999. Ocular manifestations in the follow-up of victims after sarin poisoning in Matsumoto area. *Rinsho Ganka* (*Japanese Journal Clinical Ophthalmology*) 53(4):659–663.

NRC (National Research Council). 1985. *Possible Long-Term Health Effects of Short-Term Exposure to Chemical Agents, Vol. 3. Final Report. Current Health Status of Test Subjects.* Washington, DC: National Academy Press.

Ohbu S, Yamashina A, Takasu N, Yamaguchi T, Murai T, Nakano K, Matsui Y, Mikami R, Sakurai K, Hinohara S. 1997. Sarin poisoning on Tokyo subway. *The Southern Medical Journal* 90(6):587–593.

PAC (Presidential Advisory Committee).1996. *Presidential Advisory Committee on Gulf War Veterans' Illnesses: Final Report.* Washington, DC: US Government Printing Office.

Page WF. 2003. Long-term health effects of exposure to sarin and other anticholinesterase chemical warfare agents. *Military Medicine* 168(3):239–245.

Proctor SP, Heeren T, White RF, Wolfe J, Borgos MS, Davis JD, Pepper L, Clapp R, Sutker PB, Vasterling JJ, Ozonoff D. 1998. Health status of Persian Gulf War veterans: Self-reported symptoms, environmental exposures and the effect of stress. *International Journal of Epidemiology* 27(6):1000–1010.

Reid S, Hotopf M, Hull L, Ismail K, Unwin C, Wessely S. 2001. Multiple chemical sensitivity and chronic fatigue syndrome in British Gulf War veterans. *American Journal of Epidemiology* 153(6):604–609.

Roland PS, Haley RW, Yellin W, Owens K, Shoup AG. 2000. Vestibular dysfunction in Gulf War syndrome. *Otolaryngology and Head and Neck Surgery* 122(3):319–329.

Rostker B. 2000. US demolition operations at Khamisiyah. Washington DC: Department of Defense, December 7. http://www.gulflink.osd.mil/khamisiyah_ii/.

Sekijima Y, Morita H, Yanagisawa N. 1997. Follow-up of sarin poisoning in Matsumoto. *Annals of Internal Medicine* 127(11):1042.

Shapiro SE, Lasarev MR, McCauley L. 2002. Factor analysis of Gulf War illness: What does it add to our understanding of possible health effects of deployment? *American Journal of Epidemiology* 156(6):578–585.

Simon GE, Daniell W, Stockbridge H, Claypoole K, Rosenstock L. 1993. Immunologic, psychological, and neuropsychological factors in multiple chemical sensitivity. *Annals of Internal Medicine* 119(2):97–103.

Smith TC, Gray GC, Weir JC, Heller JM, Ryan MA. 2003. Gulf War veterans and Iraqi nerve agents at Khamisiyah: Postwar hospitalization data revisited. *American Journal of Epidemiology* 158(5):457–467.

Spencer PS, McCauley LA, Lapidus JA, Lasarev M, Joos SK, Storzbach D. 2001. Self-reported exposures and their association with unexplained illness in a population-based case–control study of Gulf War veterans. *Journal of Occupational and Environmental Medicine* 43(12):1041–1056.

Storzbach D, Campbell KA, Binder LM, McCauley L, Anger WK, Rohlman DS, Kovera CA. 2000. Psychological differences between veterans with and without Gulf War unexplained symptoms. *Psychosomatic Medicine* 62(5):726–735.

Storzbach D, Rohlman DS, Anger WK, Binder LM, Campbell KA. 2001. Neurobehavioral deficits in Persian Gulf veterans: Additional evidence from a population-based study. *Environmental Research* 85(1):1–13.

Suadicani P, Ishoy T, Guldager B, Appleyard M, Gyntelberg F. 1999. Determinants of long-term neuropsychological symptoms. *Danish Medical Bulletin* 46(5):423–427.

Unwin C, Blatchley N, Coker W, Ferry S, Hotopf M, Hull L, Ismail K, Palmer I, David A, Wessely S. 1999. Health of UK servicemen who served in the Persian Gulf War. *Lancet* 353(9148):169–178.

White RF, Proctor SP, Heeren T, Wolfe J, Krengel M, Vasterling J, Lindem K, Heaton KJ, Sutker P, Ozonoff DM. 2001. Neuropsychological function in Gulf War veterans: Relationships to self-reported toxicant exposures. *American Journal of Industrial Medicine* 40(1):42–54.

Winkenwerder W. 2002. US demolition operations at Khamisiyah. Final Report. Washington, DC: Department of Defense. http://www.gulflink.osd.mil/khamisiyah_iii/.

Wolfe J, Proctor SP, Erickson DJ, Hu H. 2002. Risk factors for multisymptom illness in US Army veterans of the Gulf War. *Journal of Occupational and Environmental Medicine* 44(3):271–281.

Yokoyama K, Araki S, Murata K, Nishikitani M, Okumura T, Ishimatsu S, Takasu N. 1998a. A preliminary study on delayed vestibulo-cerebellar effects of Tokyo Subway sarin poisoning in relation to gender difference: Frequency analysis of postural sway. *Journal of Occupational and Environmental Medicine* 40(1):17–21.

Yokoyama K, Araki S, Murata K, Nishikitani M, Okumura T, Ishimatsu S, Takasu N. 1998b. Chronic neurobehavioral and central and autonomic nervous system effects of Tokyo subway sarin poisoning. *Journal of Physiology, Paris* 92(3–4):317–323.

Yokoyama K, Araki S, Murata K, Nishikitani M, Okumura T, Ishimatsu S, Takasu N, White RF. 1998c. Chronic neurobehavioral effects of Tokyo subway sarin poisoning in relation to post-traumatic stress disorder. *Archives of Environmental Health* 53(4):249–256.

Yokoyama K, Araki S, Nishikitani M, Sato H. 2002. Computerized posturography with sway frequency analysis: Application in occupational and environmental health. *Industrial Health* 40(1):14–22.

4

Conclusions

This chapter presents the committee's conclusions regarding the association between exposure to sarin or cyclosarin and various health outcomes in humans. It summarizes the literature discussed in Chapter 3 by health outcome, and discusses the biologic plausibility of an association between a given health outcome and exposure to sarin or cyclosarin on the basis of experimental animal and in vitro studies discussed in Chapter 2. As discussed in Chapter 1, human data form the main basis of the committee's conclusions. Chapter 1 contains a more detailed discussion of the committee's approach to evaluating the evidence, and the criteria for various categories of evidence are presented in Box 1-1.

A conclusion is not reached for every health outcome discussed in every article. For some health outcomes too little information is available to support a conclusion. For each relevant health outcome, epidemiology studies that examined that outcome are reviewed followed by the conclusions for that outcome and the rationale behind that conclusion.

NEUROLOGIC EFFECTS

A number of studies have evaluated the possible relationship between exposure to sarin or cyclosarin and long-lasting neurologic effects. Those outcomes have been the focus of the largest number of studies because of the neurotoxic actions of the chemicals. The studies are divided by the short-term and long-term effects of acute exposures and by the effects of chronic exposures.

Effects Following Acute High-Dose Sarin

Short-Term Effects

It is well established that high doses of sarin can have severe effects that are termed the acute cholinergic syndrome. The effects of acute poisonings from sarin have been seen in studies of servicemen who volunteered for studies of the health effects of low-dose exposure to sarin and other chemical-warfare (CW) agents (NRC, 1985; Baker and Sedgwick, 1996), a study of workers accidentally exposed to sarin (Duffy et al., 1979; Burchfiel and Duffy, 1982), and studies after terrorist attacks in Matsumoto and Tokyo, Japan (Murata et al., 1997; Nakajima et al., 1998, 1999; Yokoyama et al., 1998a,b,c).

The following conclusion was reached in the present report after a review of the literature on sarin:

> **There is sufficient evidence of a causal relationship between exposure to sarin and a dose-dependent acute cholinergic syndrome that is evident seconds to hours subsequent to sarin exposure and resolves in days to months.**

The acute cholinergic syndrome has been recognized for decades and has been documented in human studies summarized in Chapter 3. As discussed in Chapter 2, animal and mechanistic data are consistent with the effects seen in humans. The syndrome, as well as cholinergic signs and symptoms, is evident seconds to hours after exposure and usually resolves in days to months. The syndrome and the cholinergic signs and symptoms are produced by sarin's irreversible inhibition of the enzyme acetylcholinesterase. Inactivation of the enzyme that normally breaks down the neurotransmitter acetylcholine leads to the accumulation of acetylcholine at cholinergic synapses. Excess quantities of acetylcholine result in widespread overstimulation of muscles and nerves. At high doses, convulsions and death can occur.

Long-Term Effects

Many health effects are reported in the literature to persist after sarin exposure: fatigue, headache, visual disturbances (asthenopia, blurred vision, and narrowing of the visual field), asthenia, shoulder stiffness, and symptoms of posttraumatic stress disorder (PTSD). Sarin exposure has been followed by abnormal test results, of unknown clinical significance, on the digit symbol test of psychomotor performance, electroencephalographic (EEG) records of sleep, event-related potential, visual evoked potential, and computerized posturography.

Studies of servicemen who volunteered for a study of the health effects of low-dose exposure to sarin and other CW agents did not demonstrate any long-term health effects of exposure to cholinesterase inhibitors (NRC, 1985; Page,

2003). A small, noncontrolled study of British servicemen who volunteered for a study of sarin exposure showed indications of potential failure of transmission at the neuromuscular junction 2 years after exposure, but the indicators had disappeared by a year later (Baker and Sedgwick, 1996). Although the exact doses received in the US study are not known, in both the US and British studies some people experienced the acute cholinergic syndrome.

A study of workers accidentally exposed to sarin who exhibited the acute cholinergic syndrome showed changes in EEG findings, but their clinical significance is not known (Duffy et al., 1979; Burchfiel and Duffy, 1982).

Studies have followed health effects in people who exhibited the acute cholinergic syndrome after terrorist attacks in Matsumoto and Tokyo, Japan. Three years after the Matsumoto attack, fatigue, headache, and the visual disturbances asthenopia, blurred vision, and narrowing of visual field were more common among people who reported signs of the acute cholinergic syndrome than among those who lived near the sarin release site who did not have signs of the cholinergic syndrome (Nakajima et al., 1998, 1999). An English abstract also showed visual-field constriction and abnormal EEG 45 months after the attacks (Nohara et al., 1999). Some 6–8 months after the Tokyo attack, symptom-free survivors of intermediate to high exposures were impaired on only one of nine neurobehavioral tests, and significant changes in some EEG findings and postural sway tests were seen in females (Murata et al., 1997, Yokoyama et al., 1998a,b,c). Three years after the Tokyo attack, a dose-effect relationship was found in previously poisoned people on a measure of memory performance (the backward digit span test), and tapping interval for dominant hand and stabilometry measures with eyes open were affected in exposed people (Nishiwaki et al., 2001). A noncontrolled study of patients from the Tokyo attack showed ocular effects (tiredness of eyes, dim vision, and difficulty in focusing), tiredness, fatigue, stiff muscles, and headache up to 5 years after the attack (Kawana et al., 2001).

The present committee concluded that:

There is limited/suggestive evidence of an association between exposure to sarin at doses sufficient to cause acute cholinergic signs and symptoms and a variety of subsequent long-term neurological effects.

The conclusion is based on the persistent effects seen in retrospective studies, discussed above, of three exposed populations in which acute cholinergic signs and symptoms were documented as acute effects of exposure. The findings from the studies were based on comparisons with control populations. One exposed population consisted of industrial workers accidentally exposed to sarin in the United States; the other two populations were of civilians exposed during terrorism episodes in Japan. The health effects listed above were documented at least 6 months after sarin exposure, and some persisted up to 3 years, depending on the study. A review of the literature published since the preparation of *GW1* confirmed that the effects were seen in those populations, but taken together the

data were not adequate to increase confidence in the evidence to that of sufficient evidence of an association.

Effects Following Low-Level Exposures

Studies of forces deployed to the Gulf War have investigated the possible long-term neurologic effects that might be related to exposure to low levels of sarin. General neurologic effects and posttraumatic stress disorder (PTSD) are discussed separately below.

General Neurologic Effects

The most relevant studies have been conducted on US troops who were potentially exposed to sarin after munitions demolition at Khamisiyah, Iraq. There are no reports that any of those or other troops had signs of the acute cholinergic syndrome. Hospitalization studies found no difference in hospitalizations for nervous system diseases between exposed and nonexposed veterans (Gray et al., 1999; Smith et al., 2003). Studies of veterans from five states who were present when the demolition occurred showed no differences between troops who were and who were not present at Khamisiyah (McCauley et al., 2001). When those who did and those who did not witness the explosion were questioned about symptoms present 8 years after the explosion, however, those who reported witnessing the explosion had changes in memory, difficulty in sleeping, persistent fatigue, and depression (McCauley et al., 2001). It is difficult to determine if those effects are biased by the knowledge that the individuals might have been exposed to sarin. As discussed in Chapter 3, the uncertainties surrounding the exposure assessments for Khamisiyah limit the ability of those studies to provide strong evidence for the presence or absence of any associations.

A number of studies have been conducted on cohorts from the Gulf War based on analyses of self-reports of possible indicators of sarin exposure on questionnaires. People's reports that they had exposure to "chemical-warfare agents" have been associated with neurologic findings in several studies: cognitive dysfunction, depression, and fibromyalgia (Iowa Persian Gulf Study Group, 1997); major depression and anxiety (Goss Gilroy Inc., 1998); a syndrome termed "confusion–ataxia" (problems with thinking, disorientation, balance disturbances, vertigo, and impotence) (Haley and Kurt, 1997); mood, memory, and cognitive deficits (profile of mood states, tension and confusion scales, three tests of recall memory, and the WMS-R backward digit span test) (White et al., 2001); and musculoskeletal, neurologic, neuropsychologic, and psychologic symptoms (Proctor et al., 1998). In those studies, however, there is no documentation of actual exposure to chemical or biological warfare agents. In the absence of any exposure data beyond self-reports, the committee concluded that such effects could not be attributed to sarin or cyclosarin.

Other studies have not shown such effects or have shown inconsistent effects. In a study of Danish Gulf War veterans, all of whom were involved in peacekeeping or humanitarian roles after the end of the war, self-reported exposure to "nerve gas" was not significantly associated with the neuropsychologic symptoms in the Gulf War cohort (Suadicani et al., 1999). Goss Gilroy Inc. (1998) reported no association with symptoms of cognitive dysfunction, chronic fatigue, and fibromyalgia in Canadian veterans. Because the servicemen and women in those two studies were not present in the gulf or not on the ground in the gulf at the time of the Khamisiyah demolition, however, they provide little information on the possible effects of sarin.

Posttraumatic Stress Disorder

PTSD has been seen in survivors of the Matsumoto (Nohara et al., 1999; English abstract) and Tokyo (Yokoyama et al., 1998c; Kawana, 2001) sarin terrorist attacks; however, those individuals had exhibited the acute cholinergic syndrome.

McCauley et al. (2002) found deployed veterans to be more likely to have PTSD than nondeployed veterans. They did not find PTSD to be more common among Khamisiyah-exposed than Khamisiyah-nonexposed Gulf War veterans. As noted above, there were many uncertainties surrounding exposure assessments.

Studies of Gulf War veterans found PTSD associated with self-reports of potential exposures in the Gulf War (e.g., Goss Gilroy Inc., 1998; Proctor et al., 1998). Those potential exposures relating to sarin for which associations were found include self-reports of exposure to chemical-warfare agents, self-reports of hearing chemical alarms, and wearing protective gear. British veterans reported either wearing "nuclear, biological, and chemical warfare suits", "hearing chemical alarms", or having a "chemical/nerve gas attack" (Unwin et al., 1999). Exposure assessment in those Gulf War studies is weak, relying on self-reports of exposure to sarin or other CW agents. It is not clear, therefore, whether PTSD-related effects are mediated by sarin itself. Although PTSD and related neurologic effects hypothetically might be toxicologic effects mediated by sarin, they might also be emotional and physiologic responses to the psychologic trauma of chemical agent exposure, which could be actual or perceived, or by the trauma of war itself. Many of the questionnaires were administered after there was knowledge of potential exposure. Exposure to severe psychological trauma is a nonspecific cause of PTSD, according to numerous studies in military, occupational, and civilian populations (see Kessler, 2000 for review). Military and occupational groups with psychological trauma exposure show similar signs of PTSD to those with self-reported exposures that might indicate exposure to sarin. Those groups include police and other rescue workers (Weiss et al., 1995; Asukai et al., 2002) and other veteran populations (Kulka et al., 1990). As discussed in *GW1* (IOM,

2000), the *belief* in chemical-warfare exposure is sufficiently traumatic by itself that it can produce anxiety and depression symptoms in the absence of actual exposure, according to research on military trainees undergoing mock chemical-warfare exercises (Fullerton and Ursano, 1990). Further, in Canadian veterans of the Gulf War, self-reported chemical warfare agent exposure was associated with depression and anxiety symptoms, yet most Canadian veterans are unlikely to have been exposed to sarin because they were based at sea or had left the Gulf theatre at the time of the Khamisiyah demolitions (Goss Gilroy Inc., 1998). Finally, other traumatic and life-threatening exposures during the Gulf War were associated with neurological effects and could have accounted for neurological and psychiatric symptoms or diagnoses. PTSD symptoms or diagnoses were more likely in Gulf War veterans with combat exposure or injury (Baker et al., 1997; Labbate et al., 1998; Wolfe et al., 1998), with exposure to missile attack (Perconte et al., 1993), and with grave-registration duties (Sutker et al., 1994). It should also be noted that during the Khamisiyah demolitions, no chemical alarms were heard because the sarin concentration was below the sensitivity of the instruments to detect it. Chemical alarms also were triggered by substances other than chemical-warfare agents, such as organic solvents, exhaust fumes, and insecticides. Therefore, hearing chemical alarms is a poor indicator of exposure to chemical-warfare agents.

The wide range of traumatic exposures associated with PTSD prompted Veterans Administration researchers to examine, in a large population-based study of Gulf War veterans, the combined effect of what they defined as three major combat stressors: 1) "hearing chemical alarms or wearing protective gear"; 2) "direct combat duty"; and 3) "witnessed any deaths." The study found that the likelihood of developing PTSD after the war increased with increasing levels of traumatic exposure in a dose-dependent manner (Kang et al., 2003).

Conclusion

The present committee concluded that:

There is inadequate/insufficient evidence to determine whether an association does or does not exist between exposure to sarin at low doses insufficient to cause acute cholinergic signs and symptoms and subsequent long-term adverse neurological health effects.

On the basis of findings in a study of organophosphorus (OP) insecticides in nonhuman primates and in some studies of humans exposed to them, the question is raised whether there are long-term adverse health effects of exposure to low doses of sarin. Studies of low exposure of workers find that OP insecticides are associated with a higher prevalence of neurologic or psychiatric symptom reporting. Similar studies of low-dose exposure to sarin do not exist. As dis-

cussed in Appendix A, the committee does not believe that it is possible to extrapolate the long-term, low-level effects of OP insecticides to the case of sarin and cyclosarin. Although the OP insecticides and the OP nerve agents are known to share a mechanism of action underlying their acute effects (inhibition of cholinesterase), the mechanism that underlies any potential low-level effects of OP insecticides is less established. Furthermore, the committee responsible for *Gulf War and Health: Volume 2* (*GW2*; IOM, 2003) was unable to reach a consensus as to whether the evidence of those effects following exposure to OP insecticides was "inadequate/insufficient" or "limited/suggestive". The present committee reviewed articles published since the preparation of *GW2* and did not believe that any published studies definitively demonstrate those effects or lack thereof.

As discussed above, none of the studies using exposure information showed persistent neurologic effects in Khamisiyah-exposed troops compared to Khamisiyah-nonexposed troops. Because of the uncertainty in the exposure assessment models discussed previously, however, those studies do not provide strong evidence for or against the presence of neurologic effects. A number of studies of Gulf War veterans found a variety of neurological effects associated with self-reports of potential exposures in the Gulf War. Those potential exposures related to sarin for which associations have been seen include self-reports of hearing chemical alarms and wearing protective gear. As discussed earlier, those self-reports of potential indicators of exposure do not provide strong evidence of an exposure–effect relationship and, therefore, those studies provide little evidence that those effects are mediated by sarin itself. In the case of PTSD and related neurologic effects, hypothetically those effects might be toxicologic effects mediated by sarin, but the committee believes that those effects are more likely emotional and physiologic responses to psychologic trauma. Many different traumatic exposures not related to sarin were also associated with PTSD in the Gulf War veterans.

Data on experimental animals, especially from studies by Henderson et al. (2001, 2002), which were designed to mimic the potential exposures in the Gulf War, have demonstrated changes in muscarinic receptor density in specific brain areas. Those data are an important step in determining whether a biologically plausible mechanism could underlie any long-term effects of low exposure to chemical nerve agents, but more work needs to be conducted to elucidate potential mechanisms and clarify how the cellular effects are related to any clinical effects that might be seen.

Therefore, in the absence of carefully designed human studies expressly of sarin's or cyclosarin's long-term health effects at doses that do not produce acute signs and symptoms, the committee concludes that the data remain inadequate or insufficient to determine whether persistent long-term effects are associated with low-level sarin exposure.

CARDIOVASCULAR EFFECTS

Persistent cardiovascular effects have been reported after the sarin attacks in Japan and in veterans of the Gulf War. A cardiovascular effect (sudden palpitation) was noted up to 6 months after the Tokyo sarin attack (Kawana, 2001), and an English-language abstract of a study of Matsumoto attack survivors discussed electrocardiographic (ECG) effects (Nohara et al., 1999). In other studies, however, 6–8 months after the Tokyo attack no changes in ECG were evident in people who recovered from an acute poisoning episode (Murata et al., 1997, Yokoyama et al., 1998a,b,c). In a study of military deployed during the time of Khamisiyah, Smith et al. (2003) found 1 of 10 specific cardiac diagnoses (cardiac dysrhythmia) to be more frequent in the exposed than in nonexposed people. Other studies of veterans showed various cardiovascular effects, but only for deployed versus nondeployed veterans, with no analysis for exposure to sarin (McCauley et al., 2002).

The present committee concluded that:

There is inadequate/insufficient evidence to determine whether an association does or does not exist between exposure to sarin and subsequent long-term cardiovascular effects.

As discussed above, reports of persistent cardiovascular effects after the sarin attacks in Japan have been inconsistent. Only one study of military deployed during the time of Khamisiyah showed cardiovascular effects. On the basis of those data, the committee concluded that the data are inadequate or insufficient to determine whether an association exists. Furthermore, the cardiovascular effects of sarin and related compounds have not been studied to any great extent in animals to determine their biological plausibility.

OTHER HEALTH EFFECTS

A number of other health effects have been seen in people potentially exposed to sarin. Some are established disorders, and others are groups of symptoms that have been clustered into syndromes or illnesses. The presence of multisymptom illness, Gulf War illness, or unexplained illness and their relationship to possible indicators of exposure to CW agents have been studied in Gulf War veterans. Gray et al. (2002) found a case of Gulf War illness associated with "use of gas masks", Nisenbaum et al. (2000) found that responding yes to "thought biological or chemical weapons were being used" was associated with meeting the criteria for a severe or mild-to-moderate case of multisymptom illness, and Wolfe et al. (2002) found an association between high frequency of "placement on formal alert for chemical and biological warfare" and mild-to-moderate or severe multisymptom illness. Reid et al. (2001) found the prevalence of multiple chemical sensitivity to be associated with hearing chemical alarms and self-

reports of having a chemical or nerve-gas attack. The prevalence of chronic fatigue syndrome was associated with hearing chemical alarms.

There is too little information for the committee to draw conclusions on any of those health outcomes in relation to sarin exposure.

REFERENCES

Asukai N, Kato H, Kawamura N, Kim Y, Yamamoto K, Kishimoto J, Miyake Y, Nishizono-Maher A. 2002. Reliability and validity of the Japanese-language version of the impact of event scale-revised (IES-R-J): Four studies of different traumatic events. *Journal of Nervous and Mental Disease* 190(3):175–182.

Baker DJ, Sedgwick EM. 1996. Single fibre electromyographic changes in man after organophosphate exposure. *Human and Experimental Toxicology* 15(5):369–375.

Baker DG, Mendenhall CL, Simbartl LA, Magan LK, Steinberg JL. 1997. Relationship between posttraumatic stress disorder and self-reported physical symptoms in Persian Gulf War veterans. *Archives of Internal Medicine* 157(18):2076–2078.

Burchfiel JL, Duffy FH. 1982. Organophosphate neurotoxicity: Chronic effects of sarin on the electroencephalogram of monkey and man. *Neurobehavioral Toxicology and Teratology* 4(6):767–778.

Duffy FH, Burchfiel JL, Bartels PH, Gaon M, Sim VM. 1979. Long-term effects of an organophosphate upon the human electroencephalogram. *Toxicology and Applied Pharmacology* 47(1):161–176.

Fullerton CS, Ursano RJ. 1990. Behavioral and psychological responses to chemical and biological warfare. *Military Medicine* 155(2):54–59.

Goss Gilroy Inc. 1998. *Health Study of Canadian Forces Personnel Involved in the 1991 Conflict in the Persian Gulf,* Vol. 1. Ottawa, Ontario: Goss Gilroy Inc. Prepared for the Department of National Defence.

Gray GC, Kaiser KS, Hawksworth AW, Hall FW, Barrett-Connor E. 1999. Increased postwar symptoms and psychological morbidity among US Navy Gulf War veterans. *American Journal of Tropical Medicine and Hygiene* 60(5):758–766.

Gray GC, Reed RJ, Kaiser KS, Smith TC, Gastanaga VM. 2002. Self-reported symptoms and medical conditions among 11,868 Gulf War-era veterans: The Seabee Health Study. *American Journal of Epidemiology* 155(11):1033–1044.

Haley RW, Kurt TL. 1997. Self-reported exposure to neurotoxic chemical combinations in the Gulf War. A cross-sectional epidemiologic study. *Journal of the American Medical Association* 277(3):231–237.

Henderson RF, Barr EB, Blackwell WB, Clark CR, Conn CA, Kalra R, March TH, Sopori ML, Tesfaigzi Y, Menache MG, Mash DC, Dokladny K, Kozak W, Kozak A, Wachulec M, Rudolph K, Kluger MJ, Singh SP, Razani-Boroujerdi S, Langley RJ. 2001. Response of F344 rats to inhalation of subclinical levels of sarin: Exploring potential causes of Gulf War illness. *Toxicology and Industrial Health* 17(5–10):294–297.

Henderson RF, Barr EB, Blackwell WB, Clark CR, Conn CA, Kalra R, March TH, Sopori ML, Tesfaigzi Y, Menache MG, Mash DC. 2002. Response of rats to low levels of sarin. *Toxicology and Applied Pharmacology* 184(2):67–76.

IOM (Institute of Medicine). 2000. *Gulf War and Health, Volume 1: Depleted Uranium, Pyridostigmine Bromide, Sarin, and Vaccines*. Washington, DC: National Academy Press.

IOM. 2003. *Gulf War and Health, Volume 2: Insecticides and Solvents.* Washington, DC: The National Academies Press.

Iowa Persian Gulf Study Group. 1997. Self-reported illness and health status among Gulf War veterans: A population-based study. *Journal of the American Medical Association* 277(3):238–245.

Kang HK, Natelson BH, Mahan CM, Lee KY, Murphy FM. 2003. Post-traumatic stress disorder and chronic fatigue syndrome-like illness among Gulf War veterans: A population-based survey of 30,000 veterans. *American Journal of Epidemiology* 157(2):141–148.

Kawana N, Ishimatsu S, Kanda K. 2001. Psycho-physiological effects of the terrorist sarin attack on the Tokyo subway system. *Military Medicine* 166(12 Suppl):23–26.

Kessler RC. 2000. Posttraumatic stress disorder: the burden to the individual and to society. *Journal of Clinical Psychiatry* 61(Suppl 5):4–12.

Kulka RA, Schlenger WE, Fairbank JA, Hough RL, Jordan BK, Marmar CR, Weiss DS. 1990. *Trauma and the Vietnam War Generation: Report of Findings from the National Vietnam Veterans Readjustment Study.* New York: Brunner/Mazel.

Labbate LA, Cardena E, Dimitreva J, Roy M, Engel CC. 1998. Psychiatric syndromes in Persian Gulf War veterans: An association of handling dead bodies with somatoform disorders. *Psychotherapy & Psychosomatics* 67(4-5):275–279.

McCauley LA, Rischitelli G, Lambert WE, Lasarev M, Sticker DL, Spencer PS. 2001. Symptoms of Gulf War veterans possibly exposed to organophosphate chemical warfare agents at Khamisiyah, Iraq. *International Journal of Occupational and Environmental Health* 7(2):79–89.

McCauley LA, Lasarev M, Sticker D, Rischitelli DG, Spencer PS. 2002. Illness experience of Gulf War veterans possibly exposed to chemical warfare agents. *American Journal of Preventive Medicine* 23(3):200–206.

Murata K, Araki S, Yokoyama K, Okumura T, Ishimatsu S, Takasu N, White RF. 1997. Asymptomatic sequelae to acute sarin poisoning in the central and autonomic nervous system 6 months after the Tokyo subway attack. *Journal of Neurology* 244(10):601–606.

Nakajima T, Ohta S, Morita H, Midorikawa Y, Mimura S, Yanagisawa N. 1998. Epidemiological study of sarin poisoning in Matsumoto City, Japan. *Journal of Epidemiology* 8(1):33–41.

Nakajima T, Ohta S, Fukushima Y, Yanagisawa N. 1999. Sequelae of sarin toxicity at one and three years after exposure in Matsumoto, Japan. *Journal of Epidemiology* 9(5):337–343.

Nisenbaum R, Barrett DH, Reyes M, Reeves WC. 2000. Deployment stressors and a chronic multisymptom illness among Gulf War veterans. *Journal of Nervous and Mental Disease* 188(5):259–266.

Nishiwaki Y, Maekawa K, Ogawa Y, Asukai N, Minami M, Omae K. 2001. Sarin Health Effects Study Group. Effects of sarin on the nervous system in rescue team staff members and police officers 3 years after the Tokyo subway sarin attack. *Environmental Health Perspectives* 109(11):1169–1173.

Nohara M, Sekijima Y, Nakajima T, Mimura S. 1999. Ocular manifestations in the follow-up of victims after sarin poisoning in Matsumoto area. *Rinsho Ganka* (*Japanese Journal Clinical Ophthalmology*) 53(4):659–663.

NRC (National Research Council). 1985. *Possible Long-Term Health Effects of Short-Term Exposure to Chemical Agents, Vol. 3. Final Report. Current Health Status of Test Subjects*. Washington, DC: National Academy Press.

Page WF. 2003. Long-term health effects of exposure to sarin and other anticholinesterase chemical warfare agents. *Military Medicine* 168(3):239–245.

Perconte S, Wilson A, Pontius E, Dietrick A, Kirsch C, Sparacino C. 1993. Unit-based intervention for Gulf war soldiers surviving a SCUD missile attack: Program description and preliminary findings. *Journal of Traumatic Stress* 6(2):225–238.

Proctor SP, Heeren T, White RF, Wolfe J, Borgos MS, Davis JD, Pepper L, Clapp R, Sutker PB, Vasterling JJ, Ozonoff D. 1998. Health status of Persian Gulf War veterans: Self-reported symptoms, environmental exposures and the effect of stress. *International Journal of Epidemiology* 27(6):1000–1010.

Reid S, Hotopf M, Hull L, Ismail K, Unwin C, Wessely S. 2001. Multiple chemical sensitivity and chronic fatigue syndrome in British Gulf War veterans. *American Journal of Epidemiology* 153(6):604–609.

Smith TC, Gray GC, Weir JC, Heller JM, Ryan MA. 2003. Gulf War veterans and Iraqi nerve agents at Khamisiyah: Postwar hospitalization data revisited. *American Journal of Epidemiology* 158(5):457–467.

Suadicani P, Ishoy T, Guldager B, Appleyard M, Gyntelberg F. 1999. Determinants of long-term neuropsychological symptoms. *Danish Medical Bulletin* 46(5):423–427.

Sutker PB, Uddo M, Brailey K, Allain AN, Errera P. 1994. Psychological symptoms and psychiatric diagnoses in Operation Desert Storm troops serving grave registration duty. *Journal of Traumatic Stress* 7(2):159–171.

Unwin C, Blatchley N, Coker W, Ferry S, Hotopf M, Hull L, Ismail K, Palmer I, David A, Wessely S. 1999. Health of UK servicemen who served in the Persian Gulf War. *Lancet* 353(9148):169–178.

Weiss DS, Marmar CR, Metzler TJ, Ronfeldt HM. 1995. Predicting symptomatic distress in emergency services personnel. *Journal of Consulting and Clinical Psycholo*gy 63(3):361–368.

White RF, Proctor SP, Heeren T, Wolfe J, Krengel M, Vasterling J, Lindem K, Heaton KJ, Sutker P, Ozonoff DM. 2001. Neuropsychological function in Gulf War veterans: Relationships to self-reported toxicant exposures. *American Journal of Industrial Medicine* 40(1):42–54.

Wolfe J, Proctor SP, Davis JD, Borgos MS, Friedman MJ. 1998. Health symptoms reported by Persian Gulf War veterans two years after return. *American Journal of Industrial Medicine* 33(2):104–113.

Wolfe J, Proctor SP, Erickson DJ, Hu H. 2002. Risk factors for multisymptom illness in US Army veterans of the Gulf War. *Journal of Occupational and Environmental Medicine* 44(3):271–281.

Yokoyama K, Araki S, Murata K, Nishikitani M, Okumura T, Ishimatsu S, Takasu N. 1998a. A preliminary study on delayed vestibulo-cerebellar effects of Tokyo Subway sarin poisoning in relation to gender difference: Frequency analysis of postural sway. *Journal of Occupational and Environmental Medicine* 40(1):17–21.

Yokoyama K, Araki S, Murata K, Nishikitani M, Okumura T, Ishimatsu S, Takasu N. 1998b. Chronic neurobehavioral and central and autonomic nervous system effects of Tokyo subway sarin poisoning. *Journal of Physiology, Paris* 92(3-4):317–323.

Yokoyama K, Araki S, Murata K, Nishikitani M, Okumura T, Ishimatsu S, Takasu N, White RF. 1998c. Chronic neurobehavioral effects of Tokyo subway sarin poisoning in relation to post-traumatic stress disorder. *Archives of Environmental Health* 53(4):249–256.

Appendix

Organophosphorus Insecticides

Although there are not an abundant number of studies on the health effects of sarin and cyclosarin, a great deal of research, including much epidemiologic work, has been conducted on the health effects of other organophosphorus (OP) compounds that are used as insecticides. As discussed in Chapter 2, most of the effects of sarin are thought to be mediated by inhibition of acetylcholinesterase (AChE); that mechanism is common among OP compounds, as are some of the established health effects (such as the acute cholinergic syndrome). Because of the common mechanism of action, the health effects of those insecticides could provide some insight into potential health effects of sarin and cyclosarin. The committee responsible for *Gulf War and Health, Volume 2 (GW2;* IOM, 2003), reviewed the literature on OP compounds. This appendix summarizes that committee's findings and reviews relevant epidemiology studies published since the preparation of that report.

GULF WAR AND HEALTH: VOLUME 2 CONCLUSIONS

The committee responsible for *GW2*, reviewed the epidemiologic literature and concluded that there was limited/suggestive evidence of an association between chronic exposure to OP insecticides and two cancers, non-Hodgkin's lymphoma and adult leukemia. The committee also drew conclusions regarding the association between OP insecticides and a number of neurologic outcomes. This section summarizes that review and the conclusions of *GW2* with respect to OP insecticides, focusing on outcomes for which the *GW2* committee concluded that there was limited or suggestive evidence of an association, sufficient evidence of

an association, or sufficient evidence of a causal relationship between exposure to organophosphates and given health outcomes. It is important to remember, however, that not all health effects might be common and that dose and duration of exposure might play an important role in the occurrence of effects.

Cancers

Non-Hodgkin's Lymphoma

A number of well-conducted case–control studies show an increased risk of non-Hodgkin's lymphoma (NHL) associated with exposure to specific OP and carbamate insecticides (see IOM, 2003 for discussion). By design, such studies assign specific exposures to individual subjects albeit without direct workplace or environmental measurements; in these studies, increased risks were observed with exposure to OP agents and carbamates in general and malathion, diazinon, lindane, and carbaryl in particular. However, there are too few studies with exposure measurements at the individual insecticide level to draw conclusions on any specific insecticide. The increase in risk estimates, especially those related to OP insecticides and carbamates, lends support to a possible association. The associations are consistently increased across various categories of type of use or source of exposure information (self-report or proxy respondent). In addition, the studies that have examined insecticide and pesticide use in general have shown increased NHL risks. The potential for downward bias resulting from a healthy-worker effect inherent in studies with occupationally exposed cases and population controls underscores the positive results.

The *GW2* committee concluded, from its assessment of the epidemiologic literature, that there is limited/suggestive evidence of an association between chronic exposure to OP insecticides and non-Hodgkin's lymphoma.

Adult Leukemia

Although specific and accurate exposure information on insecticide use is difficult to ascertain in epidemiologic studies, most populations studied involve workers who use insecticides on a regular basis over the course of many years. Most of the studies discussed above reported an increased risk of leukemia, especially among those exposed to OP insecticides. The studies on specific OP agents—such as diazinon, dichlorvos, and malathion—and on the broader category of insecticides provided additional support for a conclusion on exposure to OP compounds. Most of the findings were of sufficient statistical power to detect a precise estimate of risk. Given that most studies included all types of leukemia

and that more specific cell types were identified in only a few of the studies, the committee focused its conclusion on adult leukemia broadly.

The *GW2* committee concluded, from its assessment of the epidemiologic literature, that there is limited/suggestive evidence of an association between chronic exposure to OP insecticides and adult leukemia.

Neurologic Effects

The committee responsible for *GW2* (IOM, 2003) reviewed the epidemiologic literature on neurologic effects of OP insecticide, focusing on studies that examined long-term effects. Four general types of neurologic effects were examined: peripheral neuropathy, neurobehavioral effects (assessed by symptom reporting or performance on validated neurobehavioral tests or batteries), neurologic diseases, and sensory effects. Almost all the studies of exposure to insecticides available to that committee focus on exposures to insecticides as a broad group, to insecticide mixtures, or to OP insecticides in particular.

Peripheral Neuropathy

Most of the Gulf War studies of peripheral neuropathy-like symptom–exposure relationships did not conduct clinical examinations or nerve-conduction studies. Instead, studies relied on analysis of symptom self-reports. Therefore, it is not clear to the *GW2* committee that veterans identified with some type of symptom-defined peripheral neuropathy actually had clinically diagnosable peripheral neuropathy, as defined by that committee.

The results of the studies were mixed. In one large, representative sample of UK veterans (Cherry et al., 2001), associations were found between Gulf War pesticide exposure and self-reports of neuropathy-like symptoms. A study (Haley and Kurt, 1997) of a single US military unit-identified symptom cluster (labeled Syndrome 3 by the investigators) found associations with government-issued insect repellent but not with brand-name repellents; moreover, a panel of neurologists who examined a subset of five subjects who exhibited Syndrome 3 was unable to arrive at any neurologic diagnosis. In another study (Proctor et al., 1998), the investigators created groups of symptoms from responses to questionnaires. Their musculoskeletal and neurologic symptom groups, which were defined as having some peripheral neuropathy-like symptoms, were both associated with self-reported exposure to “pesticides”, but there was no more specificity about the type of pesticide or the degree of exposure. The committee was unable to draw particular conclusions from those studies, because of their limitations, both study-specific and more general. The committee did combine findings from the Gulf War veterans’ studies with those from other populations as they drew conclusions from the entire body of evidence.

The body of epidemiologic evidence of an association between OP insecticides and peripheral neuropathy consists of numerous studies, but most were found by the committee to have methodologic limitations. The committee excluded several studies from consideration because of design flaws or lack of a thorough clinical examination for the diagnosis of peripheral neuropathy (Steenland et al., 1994; Ames et al., 1995; Engel et al., 1998; London and Myers, 1998). In two of the studies, the clinical examination was used only to exclude other causes of peripheral neuropathy rather than to diagnose it (Steenland et al., 1994; Ames et al., 1995); those studies were nevertheless evaluated for neurobehavioral effects because their methods were stronger for that set of outcomes.

Two of the best-designed studies (Savage et al., 1988; Steenland et al., 2000) evaluated did not find evidence of peripheral neuropathy. The other studies of peripheral neuropathy evaluated had some positive findings, but all had design limitations that weakened the validity of their findings.

The *GW2* committee concluded, from its assessment of the epidemiologic literature, that there is inadequate/insufficient evidence to determine whether an association exists between exposure to OP insecticides and peripheral neuropathy.

Neurobehavioral Effects

Neurobehavioral (NB) effects were broadly defined to include changes in cognition, mood, and behavior that are mediated by the central nervous system (CNS). Studies on NB effects that were reviewed in *GW2* are summarized in Table A-1. NB effects are measured via symptom questionnaires or validated tests. The strongest and largest of the studies demonstrate more NB impairment in OP-poisoned than in comparison workers (Savage et al., 1988; Steenland et al., 1994). A smaller study by Rosenstock and colleagues (1991) reported consistent findings. Results of one test used in all the studies reviewed here—digit-symbol, a test of visuomotor coordination—were shown to be abnormal in OP-poisoned workers. Most of those studies showed some effects on mood (such as increased anxiety) and an increase in self-reported CNS symptoms.

Those epidemiologic studies examined the most severely exposed persons. With previously poisoned persons, there is less chance of misclassification in the exposed group. However, exposure misclassification is more likely in the comparison groups because they had substantial past insecticide exposure. That might make it harder to detect differences between exposed and comparison groups. Despite those and other limitations discussed above, there is a consistent pattern of worse performance on NB testing with past OP poisoning. What is not clear is whether long-term effects on NB function are attributable solely to the OP poisoning event or to chronic exposure, inasmuch as the poisoned workers most likely had chronic exposure as well.

The *GW2* committee concluded, from its assessment of the epidemiologic literature, that there is limited/suggestive evidence of an association between exposure to OP insecticides at doses sufficient to cause poisoning (the acute cholinergic syndrome) and long-term neurobehavioral effects assessed with neurobehavioral testing and symptom reporting. The affected neurobehavioral domains include visuomotor, attention and executive functioning, motor functioning, and mood symptoms.

Four studies were evaluated to draw conclusions about long-term NB effects in persons handling OP insecticides but with no history of earlier OP poisoning. One found no adverse NB effects on the basis of test results in an exposed group with the best documentation of exposure to OP insecticides (Ames et al., 1995). However, the comparison was with friends of the exposed subjects, on whom little exposure history is available and 19% of whom had worked in agriculture, and the possibility of past insecticide or other exposures in the comparison group may have diminished the chances of finding significant differences between exposed people and referents. Another study compared an exposed group of sheep dippers with a large, objectively chosen comparison group that had no pesticide or chemical exposure (Stephens et al., 1995).

With control of confounding factors, the study found significant performance decrements in three NB tests of visuomotor (digit-symbol), motor (simple-reaction time), and cognitive (syntactic reasoning) functioning and a dose–response relationship for the latter. The clinical impact of those findings was described by the study authors as subtle and unlikely to be manifest as symptoms. The exposed group was more likely to report psychiatric symptoms, but the authors could not rule out the impact of social and economic factors on symptom reporting.

Several other studies had some positive findings, but also limitations. A study by Fiedler et al. (1997) found that the exposed group had significantly worse performance on a single test (simple-reaction time), but there was no dose–response relationship, and there was potential misclassification error in the comparison group; the authors speculated that the positive finding was by chance. Bazylewicz-Walczak et al. (1999) found abnormalities on simple-reaction time and on the aiming test, but their study measured performance on only six NB tests and was sparse on some aspects of methodology. A population-based study of Gulf War veterans found dizziness and balance symptoms related to pesticide handling, but there was no dose–response relationship or NB testing (Cherry et al., 2001).

Some of the *GW2* committee members believed that the evidence of long-term NB effects reached the level of "limited/suggestive" because they viewed the study by Stephens et al. (1995) as a high-quality study with positive findings consistent with findings from two smaller studies of less quality (Fiedler et al.,

TABLE A-1 Epidemiologic Studies of Neurobehavioral Effects of OP Insecticides Without Past History of OP Poisoning

Reference	Population: Exposed	Population: Control	Health Outcomes or Test Type
Ames et al., 1995	45 male pesticide applicators involved in California cholinesterase-monitoring program found to have 70% decrease in red-cell AChE or 60% decrease in serum AChE from baseline in records from 1985, 1988, 1989, but with no evidence of frank poisoning	90 male friends who had no history of past pesticide poisoning, past cholinesterase inhibition, or current pesticide exposure; no information on other past OP exposure, but 19% in agriculture	Eight computerized NB tests from Neurobehavioral Evaluation System (NES): mood scales, finger tapping, sustained attention, hand–eye coordination, simple-reaction time, digit-symbol, pattern memory, serial-digit learning; noncomputerized Santa Ana dexterity test, pursuit aiming; regression coefficients to compare controls, exposed provided from regression models
Stephens et al., 1995	146 sheep farmers exposed to OP in course of sheep dipping (no dipping in prior 2 months; contact by random-number selection; 69% response rate)	143 nonexposed rural quarry workers from same area, response rate 35%	Eight computer-administered NB tests, General Health Questionnaire, Subjective Memory Questionnaire

OP Insecticide Exposure	Adjustment	Results	Limitations
History of use of OP insecticides; no information on specifics, but significant enough to lower AChE enough to cause removal from work	Used multiple linear-regression models to adjust for age, grade level, language of test (Spanish or English) for NB testing. For motor coordination tests, models involving ethnicity, age, grade level, height, weight used; no difference in alcoholic drinks, cups of coffee, hours of sleep before testing	No significant differences between referents and exposed on NB tests (except serial-digit performance, in which exposed performed better than referents)	Authors state that workers expected not to have current exposure, but basis for expectation not clear; possible misclassification error because referent group may have had significant OP exposure
Retrospective exposure questionnaire; dose index (average number sheep × number dips/year × number of years using OP insecticides); urine sample for dialkylphosphates to confirm lack of exposure during previous 48 hours	Age, lifetime alcohol, smoking, computer familiarity, educational level, time of day of testing, first language; key ones included as covariates in multivariate analysis	Farmers significantly worse in tests of motor, visuomotor skills, cognition (simple-reaction time, digit-symbol, syntactic reasoning); dose–effect relationship for syntactic reasoning; farmers more symptomatic on General Health Questionnaire	Specific symptoms not reported; number of years of chronic exposure not reported

continued

TABLE A-1 Continued

Reference	Population: Exposed	Population: Control	Health Outcomes or Test Type
Fiedler et al., 1997	57 white male New Jersey fruit-tree farmers (pesticide applicators); initial response rate, 39%; no history of pesticide poisoning	23 volunteer blueberry/cranberry growers expected to have little or no exposure to pesticides (but other growers do have OP exposure); initial response rate, 14%; 20 male volunteer hardware-store owners; initial response rate, 8%	15 NB tests, including WRAT-R to estimate premorbid intellectual ability, MMPI-2
Bazylewicz-Walczak et al., 1999	26 women performing planting jobs in greenhouses and using OPs but without history of earlier poisoning	25 women not exposed to neuro-toxins; employed in kitchens, administrative jobs	Six NB tests (Polish adaptation of WHO NCTB), two symptom questionnaires (POMS, FSSQ); performed before, after pesticide application
Steenland et al., 2000	191 termiticide applicators from North Carolina registry, including 105 current applicators and eight formerly poisoned; median exposure, 1.8 years (1987–1997)	189 nonexposed referents (106 friends of exposed, 83 state employees)	Nine NB tests: seven from NES, Trails A and B; 24-item symptom questionnaire

OP Insecticide Exposure	Adjustment	Results	Limitations
Detailed exposure interview to construct lifetime exposure metric; red-cell AChE; potentially low exposures over long time because farmers were owners and family members	Covariance analysis to adjust for confounders; referent group significantly more years of education, better reading test (WRAT); reading-test score used as covariate in analyses of each NB variable	All red-cell AChE normal (but not compared with subjects' baseline, so acute OP effects less likely); simple-reaction time significantly longer in exposed than in referent and in high than low exposure; in regression analysis, exposure not correlated with reaction time	May have been some misclassification error because OP-pesticide exposure may have occurred in referent group of blueberry/cranberry growers; potential selection bias (farmers with pesticide problems did not want to volunteer)
OPs include dichlorvos, metamidophos, methidathion, pirimiphos-methyl; some carbamates, synthetic pyrethroids, dithiocarbamates; predominantly OPs measured on clothing, skin washes, air sampling during application midseason; dose "low" or below 0.010% of toxic dose; also carbamates, synthetic pyrethroids, dithiocarbamates	Groups similar in sex, age, education, residence, comparison of group characteristics (by ANOVA and chi-square tests) did not reveal significant differences between exposed, control groups	No significant changes over spraying season except increased errors in aiming test; long-term effects of exposure ("group factor"); OP-exposed had slower simple-reaction and slower hand-movement efficiency (aiming), more mood (anxiety, depression, fatigue), more CNS symptoms than referents	Limited number of NB tests, sparse detail on some aspects of methods
Chlorpyrifos, some chlordane (1987–1988)	Regression: age, race, education, current smoking, body-mass index	Past exposure only group: one NB test significant (grooved pegboard for dominant hand); 12 of 24 symptoms more prevalent than in referents	Possible selection bias due to inability to locate majority of exposed population; exposed, referents had occupational history of solvent exposure

continued

TABLE A-1 Continued

Reference	Population: Exposed	Population: Control	Health Outcomes or Test Type
London et al., 1997	163 (from original pool of 231) spray men selected from deciduous fruit farms in South Africa	84 nonspraying male laborers from farms, matched on age, educational status	Five NB tests based on WHO NCTB without POMS, FSSQ; other information-processing tests for populations with little education
Gomes et al., 1998	226 migrant farm workers who had worked for at least 2 years in United Arab Emirates; 92 unmatched new farm workers who had worked in farming in another country for at least 2 years	226 referents never occupationally exposed to pesticides, never handled pesticides for domestic use; employed as domestic workers or in shops, offices, or industry	Two NB tests: digit-symbol, aiming; questionnaire: 30-day-recall symptom checklist
Daniell et al., 1992	49 volunteer male apple orchard pesticide applicators from Washington State; three had previous episodes of pesticide poisoning	40 volunteer male slaughterhouse workers; 68% currently nonexposed referent subjects had prior work picking or trimming crops, 27% used pesticides in past	Five NB tests from NES in English, Spanish; computer-administered

OP Insecticide Exposure	Adjustment	Results	Limitations
Long-term exposure calculated with job-exposure matrix; recent exposure assessed with history, plasma cholinesterase within 10 days of NB testing	Multiple linear, logistic regression used for long-term outcomes, exposure, factors of age, education, past history of pesticide poisoning, recent OP exposure, residential exposure, number of years of exposure	Multiple regression models showed small yet significant correlation between lifetime occupational OP exposure and pursuit aiming, Santa Ana Test, one of 21 tests of information processing	NB data present on all subjects, not cases and controls separately; current exposure; cannot separate long-term from short-term effects; no clear comparison of referents or exposed; high alcohol use in all
Farm workers lived on farms, did tilling, pesticide spraying, harvesting; red-cell AChE measure (timing related to spraying not known); data on duration of exposure collected but not run in regression analysis	Referents matched by age, nationality	Farm workers had statistically more symptoms of dizziness, headache, restlessness, sleeplessness than referents and did worse on digit-symbol test, aiming test; on regression analysis, type of job was significant predictor of symptoms; farm work also predicted low scores on symbol test and aiming test, lower AChE activity; AChE predicted blurred vision	Current exposure; cannot separate long-term from short-term effects
OP pesticides, particularly azinphos-methyl; AChE measured	Stratified by language preference because of differences in educational level, other factors	No important differences between applicators and referents were found on preseason NB tests (when language preference considered); across-	No vocabulary or other tests to establish baseline CNS functioning; small comparison groups could contribute to difficulties in finding differences

continued

TABLE A-1 Continued

Reference	Population: Exposed	Population: Control	Health Outcomes or Test Type
Rodnitzky et al., 1975	23 exposed men: 12 farmers who personally apply OP to crops or animals, 11 commercial pesticide applicators; no information on how selected, must have used an OP compound within 2 weeks of testing date	23 farmers, matched for age, educational background; tested before spraying season or not involved in pesticide handling during spraying season	Five tests: memory tested by verbal-recall task, vigilance by simple-reaction time, signal-processing time, sentence-repetition subtest of Multilingual Aphasia Examination, proprioception (use of spring-loaded button, forefinger)

1997; Bazylewicz-Walczak et al., 1999). Other committee members believed that the evidence was inadequate/insufficient because the NB test findings were too subtle to reach the level of clinical significance, and only one of the NB test findings (syntactic reasoning, a test of cognition) showed a dose–response relationship. The nature of the symptom findings from a separate questionnaire was not reported. The findings from the two smaller studies were not sufficiently robust to reinforce those from Stephens et al. (1995).

The *GW2* committee was unable to reach consensus on a conclusion regarding exposure to OP insecticides at doses insufficient to cause poisoning (the acute cholinergic syndrome) and long-term neurobehavioral effects.

Neurologic Diseases

This section summarizes the review in *GW2* on the relationship between exposure to insecticides and neurologic diseases: Parkinson's disease (PD), amyo-

OP Insecticide Exposure	Adjustment	Results	Limitations
		season changes resulted in no differences except decrease in digit-symbol test (in Spanish-preference group); no correlation of any NB results with AChE	
Regular use of OP insecticides, but many used other types as well (not described) Red-cell and plasma AChE measured, but timing not reported; no comparison with baseline; comparison of group means	Referents matched for age, educational level	Exposed subjects performed as well as referent subjects on five tests; mean plasma AChE of exposed group lower than that of referent group but not below "normal"	Study of acute effects of pesticide exposure, particularly given relative inhibition of AChE in exposed group; no information or adjustment for other possible differences between referent and exposed, such as language or intelligence level

trophic lateral sclerosis (ALS), and Alzheimer's disease (AD). Studying the relationship between exposure and neurologic diseases posed methodologic challenges, including diagnostic uncertainty, presumed long latency, and concern about the reliability of self-reporting of past exposure, especially in patients with cognitive impairment or difficulty in communicating. The *GW2* committee considered only studies that specifically examined exposure to insecticides, as opposed to the broader category of pesticides, because the latter includes herbicides and fungicides. Most studies were concerned with occupational exposure rather than with residential or leisure exposure. The *GW2* committee found no case–control or cohort studies that specifically examined the relationship between insecticide exposure and multiple sclerosis.

Parkinson's Disease The six studies reviewed offered conflicting results about the relationship between insecticides and PD. Three of the studies found no association (Stern et al., 1991; Semchuk et al., 1992; Seidler et al., 1996), one found a protective effect (Hertzman et al., 1994), and two found an association between insecticides and PD. Of the two positive studies, Butterfield et al. (1993)

reported the highest odds ratios but was limited by low participation rates, which were considerably different between cases and controls. The best-designed study, Gorell et al. (1998), was hampered by its consideration of all exposures until the time of diagnosis, which could have included post-onset exposure.

The studies varied widely in reliability of their estimates of exposure. Investigators in some of the studies took more detailed residential or occupational exposure histories and attempted to determine whether higher exposure led to increased risk of PD. Results, again, were mixed: two studies showed higher odds ratios at higher exposures (Butterfield et al., 1993; Gorell et al., 1998), and two found no association with duration of exposure to insecticides (Semchuk et al., 1992; Hertzman et al., 1994). Overall, because of the potential for bias, including confounding, these studies do not provide good evidence of an association between insecticide exposure and PD.

The *GW2* committee concluded, from its assessment of the epidemiologic literature, that there is inadequate/insufficient evidence to determine whether an association exists between exposure to OP insecticides and Parkinson's disease.

Amyotrophic Lateral Sclerosis ALS is a rapidly fatal neurologic disorder characterized by progressive muscle weakness, muscle atrophy, and fasciculations. The disease is associated with degeneration of motor neurons in the spinal cord. Because ALS affects only motor neurons, the disease does not impair a person's mind, personality, intelligence, or memory. Nor does it affect a person's senses. About 20,000 people living in the United States are afflicted with ALS, and an estimated 5,000 people are diagnosed each year. ALS is most commonly diagnosed in people 40–60 years old, but younger people can also develop it. Men are affected slightly more often than women. Of all ALS cases, 90–95% are sporadic with no known risk factors, and 5–10% are inherited. Although the etiology of ALS is unknown, some epidemiologic studies have suggested a relationship between lead exposure and ALS because toxic lead concentrations can produce symptoms similar to those of ALS (Kamel et al., 2002). In North America, investigators generally use the term *ALS* in reference to three motor neuron diseases: ALS (the most common), progressive bulbar palsy (PBP), and progressive muscular atrophy (PMA). In Europe, investigators refer to ALS and classify the two other diagnoses as subtypes of the more generic term *motor neuron disease* (MND). PBP has the most rapidly fatal course, and PMA the most benign (Verma and Bradley, 2001). If the three diseases have different etiologies, differing case mixes within studies would render comparisons across studies difficult. There is no evidence that the etiologies differ, but no studies have addressed this specific issue.

The committee identified five case–control studies that examined the association between ALS and exposure to agricultural chemicals. Four did not specifically address exposure to insecticides but used broader categories, such as

"pesticides" or "agricultural chemicals", in their characterization of exposure; and they did not report associations (Granieri et al., 1981; Deapen and Henderson, 1986; Savettieri et al., 1991; Chancellor et al., 1993;). The fifth study did specifically address exposures to insecticides.

Only one peer-reviewed study (McGuire et al., 1997) expressly examined the relationship between insecticides and ALS. That study provided evidence of a relationship, but the possibility of selection bias resulting in an overestimation of effect cannot be excluded. There were no other studies with which to compare results.

The *GW2* committee concluded, from its assessment of the epidemiologic literature, that there is inadequate/insufficient evidence to determine whether an association exists between exposure to OP insecticides and amyotrophic lateral sclerosis.

Alzheimer's Disease AD is a neurodegenerative disease marked by progressive impairment in cognition and memory. It is the most common form of dementia in older people, with a prevalence of about 5% over the age of 65 years. AD is more common in women than in men. A variety of risk factors have been studied, but only age, family history, head trauma, fewer years of formal education, and presence of the apolipoprotein 4 allele show consistent results (CSHA, 1994; Hendrie, 1998). Nonsteroidal anti-inflammatory drugs and estrogen have been reported to be protective in a few studies (Paganini-Hill and Henderson, 1994; Wolfson et al., 2002). There is no evidence of a geographic gradient in incidence or prevalence. Because of the cognitive deficit in those suffering from AD, epidemiologic studies require the use of proxy respondents to obtain information on past exposure and lifestyle factors (Weiss et al., 1996).

The committee identified two studies that focused specifically on the relationship between insecticides and AD. Several other epidemiologic studies examined the relationship between the disease and pesticides but not insecticides (French et al., 1985; CSHA, 1994). Two other studies used occupational classes (such as farming) as proxies for exposure and so were too general for the committee's consideration (Amaducci et al., 1986; Schulte et al., 1996).

The two case–control studies reviewed found no association between insecticides and Alzheimer's disease (Gun et al., 1997; Gauthier et al., 2001). Other studies did not specifically examine insecticide exposure but focused on the broader category of "pesticides". The occupational studies reviewed used occupations as surrogates for exposure, but the committee was unable to determine whether exposure relevant to the Gulf War had occurred.

The *GW2* committee concluded, from its assessment of the epidemiologic literature, that there is inadequate/insufficient evidence to determine whether an association exists between exposure to OP insecticides and Alzheimer's disease.

RECENT EPIDEMIOLOGY STUDIES OF OP INSECTICIDES

Since the release of *GW2*, further research on sheep farmers and dippers has been published. Buchanan et al. (2001) characterized the exposures to OP pesticides that occur in various jobs associated with sheep dipping. Those researchers found that handling of the sheep dip concentrate was the most important predictor of exposure to OP pesticides. The second phase of that study investigated whether the repeated exposure to the OP pesticides resulted in cumulative and irreversible damage to nerves (Pilkington et al., 2001). Of an initial 995 sheep farmers invited to participate, 611 agreed to participate, 50 were followed up, 335 were suitable for the survey, and 293 of suitable farmers took the survey. Neurological outcomes were assessed using a symptoms questionnaire and quantitative sensory tests. Symptoms were reported as autonomic symptoms, sensory symptoms, and muscle weakness. Sheep dippers reported symptoms more often (19%) than non-sheep dippers (11%). Looking at cumulative exposure, both as OP dips and total number of days of dipping, the odds ratio adjusted for age, the nonadjusted odds ratios were elevated (OR, 1.15; 95% CI, 1.04–1.26 and OR, 1.13; 95% CI, 1.03–1.24 for number of days and OP dips, respectively). When adjusted for age and country, positive linear effects were seen for symptoms but not for sensory tests. Handling of the OP concentrate was significantly associated ($p = 0.005$) with symptoms and vibration threshold. Those results, however, were highly dependent on data from two highly exposed individuals.

REFERENCES

Amaducci LA, Fratiglioni L, Rocca WA, Fieschi C, Livrea P, Pedone D, Bracco L, Lippi A, Gandolfo C, Bino G, et al. 1986. Risk factors for clinically diagnosed Alzheimer's disease: A case-control study of an Italian population. *Neurology* 36(7):922–931.

Ames RG, Steenland K, Jenkins B, Chrislip D, Russo J. 1995. Chronic neurologic sequelae to cholinesterase inhibition among agricultural pesticide applicators. *Archives of Environmental Health* 50(6):440–444.

Bazylewicz-Walczak B, Majczakowa W, Szymczak M. 1999. Behavioral effects of occupational exposure to organophosphorous pesticides in female greenhouse planting workers. *Neurotoxicology* 20(5):819–826.

Buchanan D, Pilkington A, Sewell C, Tannahill SN, Kidd MW, Cherrie B, Hurley JF. 2001. Estimation of cumulative exposure to organophosphate sheep dips in a study of chronic neurological health effects among United Kingdom sheep dippers. *Occupational and Environmental Medicine* 58(11):694–701.

Butterfield PG, Valanis BG, Spencer PS, Lindeman CA, Nutt JG. 1993. Environmental antecedents of young-onset Parkinson's disease. *Neurology* 43(6):1150–1158.

Chancellor AM, Slattery JM, Fraser H, Warlow CP. 1993. Risk factors for motor neuron disease: A case-control study based on patients from the Scottish Motor Neuron Disease Register. *Journal of Neurology, Neurosurgery, and Psychiatry* 56(11):1200–1206.

Cherry N, Creed F, Silman A, Dunn G, Baxter D, Smedley J, Taylor S, Macfarlane GJ. 2001. Health and exposures of United Kingdom Gulf war veterans. Part I: The pattern and extent of ill health [comment]. *Occupational and Environmental Medicine* 58(5):291–298.

CSHA (Canadian Study of Health and Aging). 1994. Risk factors for Alzheimer's Disease in Canada. *Neurology* 44(11):2073–2080.

Daniell W, Barnhart S, Demers P, Costa LG, Eaton DL, Miller M, Rosenstock L. 1992. Neuropsychological performance among agricultural pesticide applicators. *Environmental Research* 59(1):217–228.

Deapen DM, Henderson BE. 1986. A case-control study of amyotrophic lateral sclerosis. *American Journal of Epidemiology* 123(5):790–799.

Engel LS, Keifer MC, Checkoway H, Robinson LR, Vaughan TL. 1998. Neurophysiological function in farm workers exposed to organophosphate pesticides. *Archives of Environmental Health* 53(1):7–14.

Fiedler N, Kipen H, Kelly-McNeil K, Fenske R. 1997. Long-term use of organophosphates and neuropsychological performance. *American Journal of Industrial Medicine* 32(5):487–496.

French LR, Schuman LM, Mortimer JA, Hutton JT, Boatman RA, Christians B. 1985. A case-control study of dementia of the Alzheimer type. *American Journal of Epidemiology* 121(3):414–421.

Gauthier E, Fortier I, Courchesne F, Pepin P, Mortimer J, Gauvreau D. 2001. Environmental pesticide exposure as a risk factor for Alzheimer's disease: A case-control study. *Environmental Research* 86(1):37–45.

Gomes J, Lloyd O, Revitt MD, Basha M. 1998. Morbidity among farm workers in a desert country in relation to long-term exposure to pesticides. *Scandinavian Journal of Work, Environment and Health* 24(3):213–219.

Gorell JM, Johnson CC, Rybicki BA, Peterson EL, Richardson RJ. 1998. The risk of Parkinson's disease with exposure to pesticides, farming, well water, and rural living. *Neurology* 50(5):1346–1350.

Granieri E, Rosati G, Tola R, Pinna L, Paolino E, D'Agostini G. 1981. Amyotrophic lateral sclerosis frequency in Italy. Incidence and prevalence in the province of Ferrara. *Acta Neurologica (Napoli)* 3(4):549–557.

Gun RT, Korten AE, Jorm AF, Henderson AS, Broe GA, Creasey H, McCusker E, Mylvaganam A. 1997. Occupational risk factors for Alzheimer disease: A case-control study. *Alzheimer Disease and Associated Disorders* 11(1):21–27.

Haley RW, Kurt TL. 1997. Self-reported exposure to neurotoxic chemical combinations in the Gulf War. A cross-sectional epidemiologic study [comment]. *Journal of the American Medical Association* 277(3):231–237.

Hendrie HC. 1998. Epidemiology of dementia and Alzheimer's disease. *American Journal of Geriatric Psychiatry* 6(2 Suppl 1):S3–S18.

Hertzman C, Wiens M, Snow B, Kelly S, Calne D. 1994. A case-control study of Parkinson's disease in a horticultural region of British Columbia. *Movement Disorders* 9(1):69–75.

IOM (Institute of Medicine). 2003. *Gulf War and Health: Volume 2. Insecticides and Solvents.* Washington, DC: The National Academies Press.

Kamel F, Umbach DM, Munsat TL, Shefner JM, Hu H, Sandler DP. 2002. Lead exposure and amyotrophic lateral sclerosis. *Epidemiology* 13(3):311–319.

London L, Myers JE. 1998. Use of a crop and job specific exposure matrix for retrospective assessment of long-term exposure in studies of chronic neurotoxic effects of agrichemicals. *Occupational and Environmental Medicine* 55(3):194–201.

London L, Myers JE, Nell V, Taylor T, Thompson ML. 1997. An investigation into neurologic and neurobehavioral effects of long-term agrichemical use among deciduous fruit farm workers in the Western Cape, South Africa. *Environmental Research* 73(1-2):132–145.

McGuire V, Longstreth WT Jr, Nelson LM, Koepsell TD, Checkoway H, Morgan MS, van Belle G. 1997. Occupational exposures and amyotrophic lateral sclerosis. A population-based case-control study. *American Journal of Epidemiology* 145(12):1076–1088.

Paganini-Hill A, Henderson VW. 1994. Estrogen deficiency and risk of Alzheimer's disease in women. *American Journal of Epidemiology* 140(3):256–261.

Pilkington A, Buchanan D, Jamal GA, Gillham R, Hansen S, Kidd M, Hurley JF, Soutar CA. 2001. An epidemiological study of the relations between exposure to organophosphate pesticides and indices of chronic peripheral neuropathy and neuropsychological abnormalities in sheep farmers and dippers. *Occupational and Environmental Medicine* 58(11):702–710.

Proctor SP, Heeren T, White RF, Wolfe J, Borgos MS, Davis JD, Pepper L, Clapp R, Sutker PB, Vasterling JJ, Ozonoff D. 1998. Health status of Persian Gulf War veterans: Self-reported symptoms, environmental exposures and the effect of stress. *International Journal of Epidemiology* 27(6):1000–1010.

Rodnitzky RL, Levin HS, Mick DL. 1975. Occupational exposure to organophosphate pesticides: A neurobehavioral study. *Archives of Environmental Health* 30(2):98–103.

Rosenstock L, Keifer M, Daniell WE, McConnell R, Claypoole K. 1991. Chronic central nervous system effects of acute organophosphate pesticide intoxication. The Pesticide Health Effects Study Group. *Lancet* 338(8761):223–227.

Savage EP, Keefe TJ, Mounce LM, Heaton RK, Lewis JA, Burcar PJ. 1988. Chronic neurological sequelae of acute organophosphate pesticide poisoning. *Archives of Environmental Health* 43(1):38–45.

Savettieri G, Salemi G, Arcara A, Cassata M, Castiglione MG, Fierro B. 1991. A case-control study of amyotrophic lateral sclerosis. *Neuroepidemiology* 10(5-6):242–245.

Schulte PA, Burnett CA, Boeniger MF, Johnson J. 1996. Neurodegenerative diseases: Occupational occurrence and potential risk factors, 1982 through 1991. *American Journal of Public Health* 86(9):1281–1288.

Seidler A, Hellenbrand W, Robra BP, Vieregge P, Nischan P, Joerg J, Oertel WH, Ulm G, Schneider E. 1996. Possible environmental, occupational, and other etiologic factors for Parkinson's disease: a case-control study in Germany. *Neurology* 46(5):1275–1284.

Semchuk KM, Love EJ, Lee RG. 1992. Parkinson's disease and exposure to agricultural work and pesticide chemicals. *Neurology* 42(7):1328–1335.

Steenland K, Jenkins B, Ames RG, O'Malley M, Chrislip D, Russo J. 1994. Chronic neurological sequelae to organophosphate pesticide poisoning. *American Journal of Public Health* 84(5):731–736.

Steenland K, Dick RB, Howell RJ, Chrislip DW, Hines CJ, Reid TM, Lehman E, Laber P, Krieg EF Jr, Knott C. 2000. Neurologic function among termiticide applicators exposed to chlorpyrifos. *Environmental Health Perspectives* 108(4):293–300.

Stephens R, Spurgeon A, Calvert IA, Beach J, Levy LS, Berry H, Harrington JM. 1995. Neuropsychological effects of long-term exposure to organophosphates in sheep dip. *Lancet* 345(8958):1135–1139.

Stern M, Dulaney E, Gruber SB, Golbe L, Bergen M, Hurtig H, Gollomp S, Stolley P. 1991. The epidemiology of Parkinson's disease. A case-control study of young-onset and old-onset patients. *Archives of Neurology* 48(9):903–907.

Verma A, Bradley WG. 2001. Atypical motor neuron disease and related motor syndromes. *Seminars in Neurology* 21(2):177–187.

Weiss A, Fletcher AE, Palmer AJ, Nicholl CG, Bulpitt CJ. 1996. Use of surrogate respondents in studies of stroke and dementia. *Journal of Clinical Epidemiology* 49(10):1187–1194.

Wolfson C, Perrault A, Moride Y, Esdaile JM, Abenhaim L, Momoli F. 2002. A case–control analysis of nonsteroidal anti-inflammatory drugs and Alzheimer's disease: Are they protective? *Neuroepidemiology* 21(2):81–86.