Gulf War and Health: Volume 1. Depleted Uranium, Pyridostigmine Bromide, Sarin, and Vaccines

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Gulf War and Health

Volume 1. Depleted Uranium, Sarin, Pyridostigmine Bromide, Vaccines

Carolyn E. Fulco, Catharyn T. Liverman, Harold C. Sox, *Editors*

Committee on Health Effects Associated with
Exposures During the Gulf War

Division of Health Promotion and Disease Prevention

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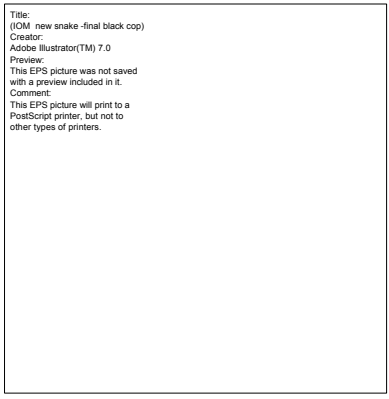
"Knowing is not enough; we must apply.
Willing is not enough; we must do."
—Goethe



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Preface

Although the Gulf War lasted but a few days, many combat troops have suffered lingering health problems that they attribute to their wartime service. Their health problems and illnesses have features in common with illnesses suffered by veterans of earlier wars, including the difficulty that their physicians have had in making a diagnosis. As yet, these illnesses remain unexplained by medical science, which has prompted some people to wonder if troops in the Persian Gulf theater were exposed to an agent or combination of agents that caused these illnesses. Research on this question continues. Another important question is whether an agent in the environment in the Persian Gulf theater could cause known conditions like heart disease or cancer.

In an effort to respond 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 associations between the agents to which Gulf War veterans may have been exposed and adverse health effects. To carry out this assignment, the IOM convened the Committee on Health Effects Associated with Exposures During the Gulf War. In planning its work, the committee contacted representatives of veterans' organizations for advice in setting its priorities for this study. The veterans and their representatives advised the committee to begin the project by studying depleted uranium, sarin, pyridostigmine bromide, and vaccination against botulinum toxin and anthrax. Reports on other agents will follow, as the Institute of Medicine and the Department of Veterans Affairs have a long-term commitment to study all of the agents to which the veterans may have been exposed. Further, the IOM will issue updated reports as new evidence appears in the scientific literature.

While the committee's work has been rewarding, it has also been quite challenging. The rewards have been largely personal. Americans owe so much to those who go to war to protect our country, yet few of us have the opportunity to do something tangible in return. The people who served on this committee had a wonderful opportunity to use their expertise to help clarify matters that are a source of concern and suffering to those who served their country in war. We felt that privilege very deeply, largely as a result of the many opportunities we had to talk with veterans who took the time and found the means to travel to Washington to advise us of their concerns. They helped us understand both the science and the human dimension of the problem that they were living with and that we had to address. Veterans, members of their families, leaders of veterans organizations, physicians, and scientists gave freely of their time. Many struggled to find words to express the suffering that they or their family members were experiencing. Our committee responded in the only way that it could—by doing our very best, individually and collectively, to carry out our assignment.

The committee sought to determine whether exposure to the agents of concern is associated with health effects in Gulf War military personnel. One of the most convincing ways to demonstrate such a relationship is to show that the magnitude of a specific health effect increases as the magnitude of the exposure increases. To achieve that goal would mean comparing the disease experience of people with differing levels of exposure to the agent. The committee soon learned that, because of extremely poor medical recordkeeping practices and limited environmental monitoring, it is not possible to document the exposure of individual Gulf War soldiers, with a few exceptions (e.g., soldiers with retained fragments of depleted uranium in their tissues). Therefore, the committee turned to studies of other populations with documented exposure to the agents of concern, including occupational-related exposure (in the case of uranium), terrorist attacks (in the case of sarin), and medical exposure (in the case of pyridostigmine bromide and vaccines). The committee can show, in some instances, that the putative agents are associated with health effects in those populations. However, the lack of information about individual Gulf War veterans' exposure to these agents means that it is not possible to show that an individual soldier experienced a dose that is associated with an increased risk of disease. Conversely, even with limited dose information, it is not possible to demonstrate that *no* health effect is related to the exposure. Possible exceptions, however, may occur when the exposure is still present, as in the case of soldiers with fragments of depleted uranium in their tissues.

The limitations imposed by poor troop monitoring and inadequate recordkeeping have been quite frustrating for the committee, as it will also be for the veterans. Yet our country has an obligation to understand illnesses that occur in those whom it asks to go to war. Past conflicts, from the Civil War to the Gulf War, have taught us that some veterans experience long-term health effects. Some of those health effects physicians will not find in a textbook of medicine. The military must lay the groundwork for understanding the health effects of future wars. It must carefully monitor the health of deployed forces and, con-

comitantly, nondeployed troops who could serve as controls. It must develop reliable methods for measuring exposure to potentially harmful agents. It must learn how to keep good medical records. For environmental exposures, the military must find ways to measure the dose experienced by individual soldiers. These tasks are technologically feasible. *For this committee, one of the most important lessons of the Gulf War is the need for accurate recordkeeping of what happens to soldiers in war.*

The nature of the evidence and of our narrowly focused charge means that our report will not satisfy everyone. We do hope that it will reassure some people. People who read the entire report will learn something about the difficulty of forming scientific conclusions based on inadequate information. We hope that our report will lead to improved troop monitoring and better medical recordkeeping practices in future military conflicts. We urgently call upon the military to collect routinely the epidemiological evidence required to understand illnesses that occur in the wake of war. We must do better next time.

Harold C. Sox, M.D.
Chair

Acknowledgments

The committee wishes to acknowledge the valuable contributions that were made to this study by the many individuals who shared their experiences and their expertise. We are especially grateful for the insight provided by many veterans, veteran's family members, representatives of veterans' organizations, and other individuals who spoke with the committee or sent in written testimony. The committee also appreciates the efforts of the Department of Defense and Department of Veterans Affairs staff in providing materials and background documents. In addition, the committee greatly benefited from the scientific expertise provided by workshop speakers, reviewers, colleagues consulted in the course of this effort, and the technical expertise provided by Marion Ehrich, Michael Katz, Michael Ryan, and Jonathan Samet. The committee also appreciates the work of the many consultants who contributed to their effort, in particular Linda Coughlin, Miriam Davis, Janice Kirsch, and Diane Mundt. The committee greatly values the guidance of John Bailar in the early phases of this study and the continued assistance of Robert Miller. Further, we are indebted to the dedication and energy provided by the Institute of Medicine staff in coordinating and steering the committee through this extensive literature review. In particular, Cathy Liverman and Carolyn Fulco provided insight and clarity of purpose, and kept us true to our task. The committee is indebted to Kathleen Stratton for her assistance in helping us negotiate our way through various difficult issues. The committee appreciates the efforts of Susan Fourt, Sandra Au, and Kysa Christie in retrieving the numerous articles required by our charge, in maintaining the databases, and for responding to all our requests for literature. A

special thanks is due to the National Library of Medicine for its assistance in accessing the extensive scientific literature. The committee also appreciates the support of the sponsor of this study, the Department of Veterans Affairs.

This report has been reviewed in draft form by individuals 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 the published report as sound as possible and to ensure that the report meets institutional standards for 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 individuals for their participation in the review of this report:

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Patrick Thomas, Bardmoor Cancer Center, Largo, Florida

M. Donald Whorton, M. Donald Whorton, Inc., Alameda, California

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 Donald R. Mattison, Medical Director, The March of Dimes Birth Defects Foundation, White Plains, New York, appointed by the Institute of Medicine, and Maureen M. Henderson, Professor Emerita, University of Washington, appointed by the NRC's Report Review Committee, who were re-

sponsible 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.

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Executive Summary

On August 2, 1990, Iraqi armed forces invaded Kuwait; within 5 days, the United States began to deploy troops to Operation Desert Shield. Intense air attacks against the Iraqi armed forces began on January 16, 1991, and opened a phase of the conflict known as Operation Desert Storm. Oil-well fires became visible by satellite images as early as February 9, 1991; the ground war began on February 24, and by February 28, 1991, the war was over. The oil fires were extinguished by November 1991. The last troops to participate in the ground war returned home on June 13, 1991. In all, approximately 697,000 U.S. troops had been deployed to the Persian Gulf area during the conflict.

Although considered an extraordinarily successful military operation with few battle casualties and deaths, veterans soon began reporting health problems that they attributed to their participation in the Gulf War. Although the majority of men and women who served in the Gulf returned to normal activities, a large number of veterans have had a range of unexplained illnesses including chronic fatigue, muscle and joint pain, loss of concentration, forgetfulness, headache, and rash.

The men and women who served in the Gulf War theater were potentially exposed to a wide range of biological and chemical agents including sand, smoke from oil-well fires, paints, solvents, insecticides, petroleum fuels and their combustion products, organophosphate nerve agents, pyridostigmine bromide (PB), depleted uranium (DU), anthrax and botulinum toxoid vaccinations, and infectious diseases, in addition to psychological and other physiological stress. Veterans have become increasingly concerned that their ill health may be related to exposure to these agents and circumstances.

In response to these concerns, the Department of Veterans Affairs (VA) approached the National Academy of Sciences and requested that the Institute of Medicine (IOM) conduct a study to evaluate the published scientific literature concerning the association between the agents to which the Gulf War veterans may have been exposed and adverse health effects. To carry out the VA charge, the IOM formed the Committee on Health Effects Associated with Exposures During the Gulf War. The committee began its deliberations in January 1999 by choosing the initial group of compounds for study. The committee decided to select the compounds of most concern to the veterans. Following meetings with representatives of different veterans' organizations, the committee decided to study the following compounds: depleted uranium, chemical warfare agents (sarin and cyclosarin), pyridostigmine bromide, and vaccines (anthrax and botulinum toxoid). Additional IOM studies will examine the remaining agents.

The committee met with veterans and leaders of veterans' organizations many times throughout the course of the study. These meetings were invaluable for the committee in providing an important perspective on the veterans' experiences and concerns. Further, ongoing discussions with and written input from veterans became an integral part of the manner in which the committee conducted the study and greatly enhanced its process.

Subsequent to the VA–IOM contract, two public laws were passed: the Veterans Programs Enhancement Act of 1998 (Public Law 105-368) and the Persian Gulf War Veterans Act of 1998 (Public Law 105-277). Each law mandated studies similar to the study already agreed upon by the VA and IOM. These laws detail several comprehensive studies on veterans' health and specify many biological and chemical hazards that may potentially be associated with the health of Gulf War veterans.

The charge to the IOM committee was relatively narrow: to assess the scientific literature regarding potential health effects of chemical and biological agents present in the Gulf War. The committee was not asked to determine whether a unique Gulf War syndrome exists, nor was it to make judgments regarding the veterans' levels of exposure to the putative agents. In addition, the committee's charge was not to focus on broader issues, such as the potential costs of compensation for veterans or policy regarding such compensation. These decisions remain the responsibility of the Secretary of Veterans Affairs. This report provides an assessment of the scientific evidence regarding health effects that may be associated with exposures to specific agents that were present in the Gulf. The Secretary may consider these health effects as the VA develops a compensation program for Gulf War veterans.

METHODOLOGY

The committee's charge was to conduct a review of the scientific literature on the possible health effects of agents to which Gulf War veterans may have been exposed. The breadth of this review included all relevant toxicological, animal, and human studies. Because only a few studies describe the veterans' exposures,

the committee reviewed studies of any human populations—including veterans—that had been exposed to the agent of concern at any dose. These studies come primarily from occupational, clinical, and healthy volunteer settings.

The committee began its task by talking with representatives of veterans' organizations, as an understanding of the veterans' experiences and perspectives is an important point of departure for a credible scientific review. The committee opened several of its meetings to veterans and other interested individuals. The committee held a scientific workshop and two public meetings. It also received information in written form from veteran organizations, veterans, and other interested persons who made the committee aware of their experiences or their health status and provided information about research. This process provided valuable information about the Gulf War experience and helped the committee to identify the health issues of concern.

The committee and staff reviewed more than 10,000 abstracts of scientific and medical articles related to the agents selected for study and then carefully examined the full text of over 1,000 peer-reviewed journal articles, many of which are described in this report. For each agent, the committee determined—to the extent that available published scientific data permitted meaningful determinations—the strength of the evidence for associations between exposure to the agent and adverse health effects. Because of the general lack of exposure measurements in veterans (with some exceptions), the committee reviewed studies of other populations known to be exposed to the agents of interest. These include uranium-processing workers, individuals who may have been exposed to sarin as a result of terrorist activity (e.g., the sarin attacks in Japan), healthy volunteers (including military populations), and clinical populations (e.g., patients with myasthenia gravis treated with PB). By studying health effects in these populations, the committee could decide, in some cases, whether the putative agents could be associated with adverse health outcomes. The committee's judgments have both quantitative and qualitative aspects, and reflect the evidence and the approach taken to evaluate that evidence. The committee's methodology draws from the work of previous IOM committees and their reports on vaccine safety (IOM, 1991, 1994a), herbicides used in Vietnam (IOM, 1994b, 1996, 1999), and indoor pollutants related to asthma (IOM, 2000).

The committee adopted a policy of using only peer-reviewed published literature to form its conclusions. It did not collect original data or perform any secondary data analysis. Although the process of peer review by fellow professionals—which is one of the hallmarks of modern science—is the best assurance that a study has reached valid conclusions, peer review does not guarantee the validity or generalizability of a study. Accordingly, committee members read each research article critically. The committee used only peer-reviewed publications in forming its conclusions about the degree of association between exposure to a particular agent and adverse health effects. However, this report describes some non-peer-reviewed publications, which provided background information for the committee and raised issues that will require further research. In their evaluation of individual research articles, committee members

considered several important issues, including the quality of the study; its relevance; issues of error, bias, and confounding; the diverse nature of the evidence; and the study population.

The committee classified the evidence for association between exposure to a specific agent and a health outcome into one of five previously established categories. The categories closely resemble those used by several IOM committees that evaluated vaccine safety (IOM, 1991, 1994a), herbicides used in Vietnam (IOM, 1994b, 1996, 1999), and indoor pollutants related to asthma (IOM, 2000). Although the categories imply a statistical association, the committee had sufficient epidemiologic evidence to examine statistical associations for only one of the agents under study (i.e., depleted uranium); the epidemiologic evidence for the other agents examined (i.e., sarin, pyridostigmine bromide, and anthrax and botulinum toxoid vaccines) was very limited. Thus, the committee based its conclusions on the strength and the coherence of the data in the available studies. In many cases, these data distinguished differences between transient and long-term health outcomes related to the dose of the agent. Based on the literature, it became incumbent on the committee to similarly specify the differences between dose levels and the nature of the health outcomes. This approach led the committee to reach conclusions about long- and short-term health effects, as well as health outcomes related to the dose of the putative agents. The final conclusions represent the committee's collective judgment. The committee endeavored to express its judgments as clearly and precisely as the available data allowed. The committee used the established categories of association from previous IOM studies, because they have gained wide acceptance for more than a decade by Congress, government agencies, researchers, and veteran groups.

- *Sufficient Evidence of a Causal Relationship.* Evidence is sufficient to conclude that a causal relationship exists between the exposure to a specific agent and a health outcome in humans. The evidence fulfills the criteria for sufficient evidence of an association (below) and satisfies several of the criteria used to assess causality: strength of association, dose–response relationship,[1] consistency of association, temporal relationship, specificity of association, and biological plausibility.
- *Sufficient Evidence of an Association*. Evidence is sufficient to conclude that there is a positive association. That is, a positive association has been observed between an exposure to a specific agent and a health outcome in human studies in which chance, bias, and confounding could be ruled out with reasonable confidence.
- *Limited/Suggestive Evidence of an Association*. Evidence is suggestive of an association between exposure to a specific agent and a health outcome in

[1]A dose–response relationship refers to the finding of a greater health effect (response) with higher doses of an agent.

humans, but is limited because chance, bias, and confounding could not be ruled out with confidence.

- *Inadequate/Insufficient Evidence to Determine Whether an Association Does or Does Not Exist*. The available studies are of insufficient quality, consistency, or statistical power to permit a conclusion regarding the presence or absence of an association between an exposure to a specific agent and a health outcome in humans.
- *Limited/Suggestive Evidence of No Association*. There are several adequate studies covering the full range of levels of exposure that humans are known to encounter that are mutually consistent in *not* showing a positive association between exposure to a specific agent and a health outcome at any level of exposure. A conclusion of no association is inevitably limited to the conditions, levels of exposure, and length of observation covered by the available studies. In addition, the possibility of a very small elevation in risk at the levels of exposure studied can never be excluded.

These five categories describe different strengths of association, with the highest level being sufficient evidence of a causal relationship between exposure to a specific agent and a health outcome. The criteria for each category sound a recurring theme: An association is more likely to be valid to the extent that the authors reduced common sources of error in making inferences—chance variation, bias in forming a study cohort, and confounding. Accordingly, the criteria for each category express varying degrees of confidence based upon the extent to which it has been possible to exclude these sources of error. To infer a causal relationship from a body of observational evidence, the committee relied on long-accepted criteria for assessing causation in epidemiology (Hill, 1971; Evans, 1976). The following sections provide a discussion and conclusions regarding the putative agents (DU, PB, sarin, and vaccines).

DEPLETED URANIUM

Depleted uranium is a by-product of the enrichment process used to make reactor-grade uranium. Natural uranium is considered a low-level radioactive element. Because of the different percentages of uranium isotopes, the specific activity (a measure of radioactivity) of depleted uranium (14.8 mBq/μg) is 40 percent lower than that of naturally occurring uranium (25.4 mBq/μg) and considerably lower than that of enriched uranium (approximately 1,750 mBq/μg) (Harley et al., 1999). However, the chemical properties of depleted uranium are the same as those of the enriched and naturally occurring forms.

The U.S. military used depleted uranium in the Gulf War for offensive and defensive purposes (OSAGWI, 1998). Heavy armor tanks had a layer of depleted uranium armor to increase protection. Depleted uranium was also used in kinetic energy cartridges and ammunition rounds. U.S. personnel were exposed to depleted uranium as the result of friendly fire incidents, cleanup operations, and

accidents (including fires). DU-containing projectiles struck 21 Army combat vehicles (OSAGWI, 1998). After the war, assessment teams and cleanup and recovery personnel may have had contact with DU-contaminated vehicles or DU munitions. In June 1991, a large fire, which occurred in Camp Doha near Kuwait City, led to a series of blasts and fires that destroyed combat-ready vehicles and DU munitions. Nearby troops and cleanup crews may have been exposed to DU-containing dust or residue. Other troops may have been exposed through contact with damaged vehicles or inhalation of DU-containing dust (Fahey, 2000).

The primary routes of exposure to uranium for humans are through ingestion or inhalation; the effects of dermal exposure and embedded fragments have also been studied. The amount of uranium retained in the body depends on the solubility of the uranium compounds to which the individual is exposed. Inhaled insoluble uranium concentrations may remain within the pulmonary tissues, especially the lymph nodes, for several years. Ingested uranium is poorly absorbed from the intestinal tract.

Conclusions on the Health Effects of Depleted Uranium

Although depleted uranium is the form of uranium that was present in the Gulf War, there are only a few studies of its health effects. Therefore, the committee studied the health effects of natural and processed uranium in workers at plants that processed uranium ore for use in weapons and nuclear reactors. The literature on uranium miners and on populations exposed to external radiation is largely not relevant to the study of uranium because the primary exposures of these populations were to other sources of radiation (e.g., radon progeny or gamma radiation). While studies of uranium processing workers are useful, these studies have several shortcomings. Although several studies involved tens of thousands of workers, even these studies were not large enough to identify small increases in the risk of uncommon cancers. Few studies had access to consistent, accurate information about individual exposure levels. Further, in these industrial settings, the populations could have been exposed to other radioisotopes (e.g., radium ore, thorium) and to a number of industrial chemicals that may confound health outcomes. Finally, no studies had reliable information about cigarette smoking, which may also confound outcomes of lung cancer. However, these cohorts of uranium processing workers are an important resource, and the committee encourages further studies that will provide progressively longer follow-up, improvements in exposure estimation, and more sophisticated statistical analyses.

Lung Cancer

Lung cancer mortality has been the focus of attention in many cohort studies of workers employed in the uranium processing industry. Many of these studies were large and had a long period of follow-up. Lung cancer mortality

was not increased among occupationally exposed persons in most of these cohorts. The strongest studies used internal controls, used multivariate analysis to adjust for possible confounders, had at least 30 years of follow-up, and measured the cumulative radiation exposure of individual workers.

In a large study of employees at Oak Ridge, Tennessee, uranium processing and research facilities (Frome et al., 1990), the entire group experienced a small increase in lung cancer mortality. Despite its shortcoming in measuring radiation exposure, the committee felt the Frome study was important because of its large size and multivariate analysis. The analysis showed that radiation exposure was not associated with lung cancer mortality. It also demonstrated the relative importance of several confounders. Socioeconomic status strongly predicted lung cancer risk. The study by Dupree and colleagues (1995) combined data from four separate studies and utilized quantitative estimates of individual cumulative exposures to uranium to form a dose–response analysis. The large number of cases of deaths from lung cancer (787) made it possible for Dupree and colleagues to perform a detailed dose–response analysis, while adjusting for confounders. This study found that the dose–response analysis did not suggest any increase in lung cancer risk up to 25 cGy. Above this level, there were too few cases to draw any conclusions. The strongest suggestion of an association with lung cancer appeared in the recent report by Ritz (1999), in which large and statistically significant increases in lung cancer mortality occurred in the small group of workers with a cumulative internal dose of 200 mSv or more. The committee viewed this finding with caution because the subgroup with the elevated risk had only three cases of lung cancer and because the author could not adjust for cigarette smoking, which had been an important factor in the Dupree study. Nevertheless, the data based on the well-characterized exposure levels in this study do suggest that after controlling for external dose, internal doses up to 200 mSv are not associated with excess risk of lung cancer.

The committee concludes that there is limited/suggestive evidence of no association between exposure to uranium and lung cancer at cumulative internal dose levels lower than 200 mSv or 25 cGy. However, there is inadequate/insufficient evidence to determine whether an association does or does not exist between exposure to uranium and lung cancer at higher levels of cumulative exposure.

Renal Function

Although uranium is a heavy metal that can cause transient renal dysfunction, the preponderance of evidence indicates little or no clinically important renal effects of exposure to uranium. A few studies have shown functional changes in renal function (Lu and Zhao, 1990; Zamora et al., 1998), but the number of cases has been quite small. Perhaps the strongest evidence is the absence of kidney damage in workers who had been exposed to high levels of

soluble uranium compounds and in veterans exposed to DU from embedded shrapnel. Kidney function was normal in Gulf War veterans with embedded DU fragments years after exposure, despite urinary uranium concentrations up to 30.74 μg/g creatinine (McDiarmid et al., 2000).

The committee concludes that there is limited/suggestive evidence of no association between exposure to uranium and clinically significant renal dysfunction.

Other Health Outcomes

The information on other health outcomes in humans comes from epidemiologic studies of uranium processing workers and case reports of workers or other individuals accidentally exposed to large doses of uranium compounds. While the studies did not suggest that uranium has adverse health effects, the studies were of insufficient quality, consistency, or statistical power to permit a conclusion regarding the presence or absence of an association in humans.

The committee concludes that there is inadequate/insufficient evidence to determine whether an association does or does not exist between exposure to uranium and the following health outcomes: lymphatic cancer; bone cancer; nervous system disease; nonmalignant respiratory disease; or other health outcomes (gastrointestinal disease, immune-mediated disease, effects on hematological parameters, reproductive or developmental dysfunction, genotoxic effects, cardiovascular effects, hepatic disease, dermal effects, ocular effects, or musculoskeletal effects).

SARIN

Sarin is a highly toxic nerve agent produced for chemical warfare. It was synthesized in 1937 in Germany in a quest for improved insecticides (Somani, 1992). Although its battlefield potential was soon recognized, Germany refrained from using its stockpiles during World War II. Sarin's first military use did not occur until the Iran–Iraq conflict in the 1980s (Brown and Brix, 1998).

High-level exposures to sarin can be fatal within minutes to hours. In vapor or liquid form, sarin can be inhaled or absorbed, respectively, across the skin, eyes, or mucous membranes (Stewart and Sullivan, 1992). Because of its extreme potency, "high" sarin exposure for humans is quite low: Exposure to as little as 100 mg across the skin, or 50–100 mg/min/m^3 by inhalation, is lethal to 50 percent of exposed individuals (Somani, 1992).

Sarin, or isopropyl methylphosphonofluoridate, is a member of a class of chemicals known as organophosphorus esters (or organophosphates). A few highly toxic members of this large class are chemical warfare agents, but most are insecticides (Lotti, 2000). The drug pyridostigmine bromide is pharmacologically

similar to sarin and other organophosphates, but it is a member of a different chemical class, the carbamates. Both PB and sarin exert their effects by binding to and inactivating the enzyme acetylcholinesterase (AChE).[2] The binding of sarin to AChE is irreversible, whereas the binding of PB to AChE is reversible.

In March 1991, during the cease-fire period, troops from the U.S. 37th and 307th Engineering Battalions destroyed enemy munitions throughout the occupied areas of southern Iraq (PAC, 1996). One of the sites destroyed was a large storage complex at Khamisiyah, Iraq, consisting of more than 100 bunkers, which contained stacks of 122-mm rockets loaded with sarin and cyclosarin[3] (Committee on Veterans' Affairs, 1998). U.S. troops performing demolitions were unaware of the presence of nerve agents. In October 1991, inspectors from the United Nations Special Commission on Iraq (UNSCOM) first confirmed the presence of a mixture of sarin and cyclosarin (Committee on Veterans' Affairs, 1998). At the time of the demolition, there were no medical reports by the U.S. Army Medical Corps of military personnel with signs and symptoms of acute exposure to sarin (PAC, 1996). Further, a 1997 survey mailed by the Department of Defense (DoD) to 20,000 troops within a 50-mile radius of Khamisiyah found that more than 99 percent of respondents (n = 7,400) reported no acute cholinergic effects (CIA–DoD, 1997). Nevertheless, low-level exposure could have occurred without producing acute cholinergic effects.

Conclusions on the Health Effects of Sarin

The committee reached the following conclusions after reviewing the literature on sarin. The committee was unable to formulate any conclusions about cyclosarin because of the paucity of toxicological and human studies.

The committee concludes that 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.

In humans, exposure to high doses of sarin produces a well-characterized acute cholinergic syndrome. This syndrome, as evidenced by acute cholinergic signs and symptoms, is evident seconds to hours after exposure and usually resolve in days to months. The syndrome is produced by sarin's irreversible inhi-

[2]AChE is an enzyme necessary to remove acetylcholine (ACh). ACh transmits nerve signals at the cholinergic neuromuscular junction or synapses in the central nervous system. Anticholinesterase agents inhibit (inactivate) AChE, resulting in an accumulation of acetylcholine. The accumulation repetitively activates the ACh receptors, resulting in exaggerated responses of the organ (e.g., excess salivation).

[3]Cyclosarin is an organophosphate nerve agent. The committee examined the literature on this agent but found a very limited amount of information available on the health effects of this compound.

bition of AChE. Inactivation of this enzyme, which 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.

The committee concludes that 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.

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

These conclusions are based on retrospective controlled studies of three different exposed populations who experienced acute cholinergic signs and symptoms after exposure to sarin. One population consisted of industrial workers accidentally exposed to sarin in the United States; the other two populations were 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 a maximum of 3 years, depending on the study. Whether the health effects noted above persist beyond the 3 years has not been studied.

The committee concludes 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 health effects.

On the basis of positive findings in a study of nonhuman primates and studies of humans exposed to organophosphate insecticides, it is reasonable to hypothesize that long-term adverse health effects can occur after exposure to low levels of sarin. Studies of industrial workers exposed to low levels of organophosphate insecticides consistently show a higher prevalence of neurological and/or psychiatric symptom reporting. However, there are no well-controlled studies of long-term health effects in humans exposed to sarin at doses that do not produce acute signs and symptoms.

PYRIDOSTIGMINE BROMIDE

Pyridostigmine bromide was used during the Gulf War as a pretreatment for exposure to nerve agents. It has been used for more than 40 years in the routine treatment of myasthenia gravis and may be used following surgery in the reversal of neuromuscular blockade (Williams, 1984).

PB, a reversible cholinesterase inhibitor, was synthesized in 1945 by Hoffman-La Roche Laboratories in Switzerland and is sold under the trade name Mestinon bromide (Williams, 1984). PB is one of the quaternary ammonium anticholinesterase compounds, which generally do not penetrate cell membranes. Compounds in this category are poorly absorbed from the gastrointestinal tract and are excluded by the blood–brain barrier (Williams, 1984; Goodman et al., 1996).

Mestinon was approved by the Food and Drug Administration (FDA) in 1955 as safe for the treatment of myasthenia gravis. The FDA also approved an injectable form known as Regenol for reversing the effects of some anesthetic formulations (Rettig, 1999). In the treatment of myasthenia gravis, the average oral dose is 120–600 mg per day (in divided doses); however, the size and frequency of the dose must be adjusted to the needs of the individual patient (*Physicians' Desk Reference*, 2000). The drug is poorly absorbed after oral administration, and peak plasma levels occur 2 to 3 hours after oral dosing. The drug is eliminated almost exclusively via the kidneys in the urine (Williams, 1984).

Side effects of PB are generally related to the large doses given to myasthenics; in surgical patients, adverse reactions are controlled by simultaneous administration of atropine (Williams, 1984). The acute cholinergic side effects of PB are due to stimulation of muscarinic or nicotinic receptors by increased acetylcholine (ACh). Muscarinic reactions include nausea, vomiting, diarrhea, abdominal cramps, increased peristalsis, increased salivation, increased bronchial secretions, miosis, and heavy perspiration. Nicotinic effects are chiefly muscle cramps, fasciculations, and weakness (Williams, 1984).

PB binds reversibly to AChE and prevents the enzyme from binding irreversibly with nerve agents. PB pretreatment is used by the military to obtain 10–20 percent inhibition of whole-blood AChE (Hubert and Lison, 1995). PB is not an antidote and has no value when administered after nerve agent exposure. It is not a substitute for atropine or 2-pralidoxime chloride; rather, it enhances their efficacy (Madsen, 1998).

The DoD reported that 5,328,710 doses of PB were fielded and estimated that approximately 250,000 personnel took PB during the Gulf War. It was supplied as a 21-tablet blister pack; the dosage prescribed was one 30-mg tablet every 8 hours. Variation in use occurred, however, because it was self-administered and was to be taken only when ordered by the unit commander (PAC, 1996). Thus, veterans' actual exposure to PB is not known, and there are few examples of documentation in either individual health records or unit records (PAC, 1996).

Conclusions on the Health Effects of Pyridostigmine Bromide

A large number of clinical studies have reported that PB causes acute transient cholinergic effects in normal volunteers, patients given PB as a diagnostic test of hypothalamic pituitary function, and myasthenia gravis patients treated with the drug for extended periods. When used as a diagnostic test, PB is generally administered as a single oral 30- to 180-mg dose, which produces acute transient cholinergic symptoms in a minority of patients and normal volunteers. Within several hours of ingesting PB, 25 percent of subjects experience abdominal symptoms (cramps, increased digestive sounds, pain, diarrhea, and nausea), and 10 percent have muscular symptoms (skeletal muscle and tongue fasciculations sometimes accompanied by dysarthria) that typically last 1–2 hours (see, for example, Ross et al., 1987; Ghigo et al., 1990a,b,c, 1996a,b; Giustina et al., 1990, 1991; Murialdo et al., 1991, 1993; Bellone et al., 1992; O'Keane et al., 1992, 1994; Cordido et al., 1995; Yang et al., 1995; Coiro et al., 1998). The symptoms are usually mild, transient, and tolerable; seldom require medical intervention; and are not accompanied by central nervous system symptoms. Although the studies summarized in this report did not show a relationship between increasing dose and more severe side effects, none was designed specifically to demonstrate a dose–response relationship. There is, however, a trend toward a greater rate of symptoms at higher PB doses among subjects given several different doses in the same study.

The main therapeutic use of PB is to control muscle weakness in myasthenic patients; the daily doses of PB usually range from 120 to 600 mg. About one-third of those who take PB have one or more side effects, which are usually mild. The most common symptoms are gastrointestinal in origin. A few patients experience other cholinergic symptoms such as hypersalivation, increased perspiration, urinary urgency, increased bronchial secretion, and blurred vision. Patients seldom stop taking the drug because of side effects.

During the Gulf War, acute accidental poisoning with PB in doses ranging from 390 to 900 mg resulted in mild-to-moderate cholinergic symptoms occurring within several minutes of ingestion and lasting up to 24 hours. Patients typically developed muscarinic effects (e.g., abdominal cramps, diarrhea, nausea, hypersalivation, vomiting), urinary incontinence, and transient muscle fasciculation and weakness. The effects were self-limited and were well tolerated.

The most extensive information available on the acute effects of PB comes from studies of its use for diagnosis of growth hormone deficiency and its therapeutic use for myasthenia gravis. The doses of PB in these applications are higher than those used for prophylaxis during the Gulf War, yet these studies consistently indicate that PB is safe and effective in clinical applications. Side effects are predominantly gastrointestinal and muscular, do not last long, and have no long-term residual effects.

Results from other human studies, in both clinical and healthy volunteer populations, report the same gastrointestinal and muscular side effects, which

are transient and characteristically mild. Idiosyncratic reactions occur at a much lower rate.

The committee concludes that there is sufficient evidence of an association between PB and transient acute cholinergic effects in doses normally used in treatment and for diagnostic purposes.

Since unexplained Gulf War-related illnesses have been chronic, possible long-term effects of PB are of great interest. There are no reports of chronic toxicity related to human PB exposure in clinical or military populations. Haley and Kurt (1997) suggested that unexplained Gulf War-related symptoms could be a unique manifestation of organophosphate-induced delayed neuropathy associated with PB exposure alone or in combination with other wartime exposures, in the absence of acute symptoms of organophosphate toxicity. There is evidence that some AChE inhibitors may be associated with chronic neurological changes. Haley and Kurt provide evidence that a small number of ill Gulf War veterans have neurological impairment compared to a small number of well veterans from the same unit. The committee felt that the validity of this association, and the possible causal relationship between PB and the neurological findings, are uncertain. Among the reasons for withholding judgment are the large potential for selection and information biases[4] in this study population, the lack of a nondeployed comparison group, and the lack of clinical validity in the measures of neurological damage. Haley and Kurt's hypothesis requires further investigation.

Haley and Kurt (1997) have also suggested that chronic neuropsychological syndromes derived from factor analysis are linked to acute responses to administration of PB. The evidence that they present has several shortcomings. The major limitation was the lack of comparable studies in a nondeployed group of veterans. There is uncertainty about how the authors selected, administered, and interpreted the neuropsychological tests. The study population consisted of self-selected individuals who replied to a survey (41 percent of the battalion). The data on exposure to PB were self-reports of events that had occurred many years before.

The epidemiologic data do not provide evidence of a link between PB and chronic illness in Gulf War veterans. Most epidemiologic studies of Gulf War veterans have focused on whether a unique Gulf War syndrome exists and defining its characteristics. Only two epidemiologic studies specifically investigated the possible association of PB use and chronic symptoms among Gulf War veterans (Haley and Kurt, 1997; Unwin et al., 1999). This summary has already noted the limitations of the small, selected population studied by Haley and colleagues. Based on factor analysis, they defined three syndromes associated with

[4]Selection bias can occur in the recruitment of study subjects to a cohort when the study and control groups differ from each other by a factor, often unknown, that is likely to affect the results. Information bias results from the way in which the data are collected (e.g., measurement errors, imprecise measurement, misdiagnosis). Bias may also result from misclassification of study subjects with respect to the outcome variable.

Gulf War service. These factor-derived syndromes were not associated with taking PB or with the dose of PB. Haley and Kurt found an association between two of the three syndromes and self-reported symptoms that are consistent with adverse effects of PB. Because the study cohort was not assembled from a random sample of Gulf War veterans, this apparent association may be the result of inadvertent selection for veterans with both adverse health syndromes and adverse effects of PB. The evidence is not strong enough to conclude that an association exists between Gulf War illnesses and side effects of PB. In the second epidemiologic study (Unwin et al., 1999), all exposures studied (PB, diesel or petrochemical fumes, oil fire smoke, viewing dismembered bodies, etc.) showed an association of similar magnitude with adverse symptoms in U.K. servicemen. The lack of specificity of the association between the type of exposure and symptoms suggests that PB itself is not the cause of the symptoms. Recall bias and reporting bias[5] may explain this finding. Thus, neither of these two studies provides good evidence for a specific association between PB and chronic adverse health effects.

The committee concludes that there is inadequate/insufficient evidence to determine whether an association does or does not exist between PB and long-term adverse health effects.

VACCINES

During the Gulf War, a number of different immunobiologics (e.g., cholera, meningitis, rabies, tetanus, and typhoid vaccines) were sent to the war theatre to protect military personnel against potential exposures to biological threats (Committee on Veterans' Affairs, 1998). Concerns about Iraq's offensive biological warfare capabilities led to the decision that available vaccines should be utilized as preventive measures against biological warfare agents. The military sent approximately 310,000 doses of FDA-licensed anthrax vaccine to the Gulf War theatre, and it is estimated that 150,000 U.S. troops received at least one anthrax vaccination (Christopher et al., 1997; Committee on Veterans' Affairs, 1998). Approximately 137,850 doses of botulinum toxoid were sent to the Gulf, and it is estimated that 8,000 military personnel were vaccinated (Committee on Veterans' Affairs, 1998). However, medical records from the Gulf War contain little or no information about who received these vaccines, how frequently the vaccines were administered, or the timing of vaccinations relative to other putative exposures (OSAGWI, 1999).

[5]Common sources of information bias are due to the inability of study subjects to accurately recall the circumstances of the exposure (recall bias) or to the likelihood that one group more frequently reports what it remembers than another group (reporting bias).

Anthrax Vaccine

The primary use of the anthrax vaccine in humans was initially for the protection of occupationally exposed individuals (e.g., persons working with animal hair or hide, including goat hair mill workers, tannery workers, and veterinarians). Protective antigen, one of the three toxin proteins produced by the anthrax bacillus, is the immunogenic component of both the U.S. and the U.K. vaccines. The U.S. vaccine is an aluminum hydroxide-adsorbed cell-free culture filtrate of an unencapsulated anthrax strain (Pile et al., 1998). Product licensure for Anthrax Vaccine Adsorbed was granted on November 10, 1970. It is estimated that 68,000 doses of the U.S. anthrax vaccine were distributed from 1974 to 1989, 268,000 doses in 1990, and 1.2 million doses from 1991 to July 1999 (Ellenberg, 1999). The exact number of people who received the vaccine is not known. The current dosing schedule is 0.5 ml administered subcutaneously at 0, 2, and 4 weeks and 6, 12, and 18 months, followed by yearly boosters.

In December 1997, the Secretary of Defense announced that all U.S. military forces would receive anthrax vaccinations for protection against the threat of biological warfare. The Anthrax Vaccine Immunization Program began vaccinations in March 1998.

Conclusions on the Health Effects of the Anthrax Vaccine

There is a paucity of published peer-reviewed literature on the safety of the anthrax vaccine. Brachman and colleagues (1962) conducted the only randomized clinical trial of vaccination with a protective antigen anthrax vaccine.[6] The clinical trial was conducted among eligible workers at four goat hair processing mills in which some raw materials were contaminated by anthrax bacilli. Participants were examined 24 and 48 hours following each vaccination to assess both local and systemic reactions to the vaccine. There were no reports of subsequent active or passive surveillance for possible adverse effects beyond 48 hours after each vaccination (however, there was further monitoring for the vaccine's efficacy). The typical reaction is described as a ring of erythema (1–2 cm in diameter) at the injection site, with local tenderness that lasted 24–48 hours. Some subjects (a number was not given) reported more extensive edema, erythema (more than 5 cm in diameter), pruritus, induration, or small painless nodules at the injection site (lasting up to several weeks). Twenty-one individuals had moderate local edema that lasted up to 48 hours. The only systemic reactions were reported in two individuals (0.9 percent of the actively vaccinated subjects) who experienced "malaise" lasting 24 hours following vaccination. The study notes that three individuals who received the placebo (0.1 percent

[6]Although the vaccine used in this study was similar to the vaccine currently available in the United States in that it was a protective antigen vaccine, the manufacturing process has since changed and a different strain of anthrax bacillus is now used (GAO, 1999).

alum) had mild reactions. However, studies of the anthrax vaccine have not used active surveillance to systematically evaluate long-term health outcomes. Unfortunately, this situation is typical for all but a few vaccines.

The committee concludes that there is sufficient evidence of an association between anthrax vaccination and transient acute local and systemic effects (e.g., redness, swelling, fever) typically associated with vaccination.

The committee concludes that there is inadequate/insufficient evidence to determine whether an association does or does not exist between anthrax vaccination and long-term adverse health effects.

Botulinum Toxoid

Botulinum toxins, known primarily for causing cases of foodborne botulism, are produced by the anaerobic bacterium *Clostridium botulinum*. Different strains of the bacillus produce seven distinct botulinum toxins (A–G). These toxins are among the most toxic compounds per body weight of agent, with an LD_{50} of 0.001 μg/kg in mice (USAMRIID, 1996).

Work on modifying the botulinum toxin to the nontoxic form of a toxoid began in 1924. A bivalent toxoid (for serotypes A and B) was developed in the United States in the 1940s. Further research led to a pentavalent toxoid (serotypes A–E) first produced in large lots by Parke, Davis, and Company in 1958 under contract to the U.S. Army (Anderson and Lewis, 1981). The current botulinum toxoid vaccine, a pentavalent toxoid (serotypes A–E), is in Investigational New Drug status. The toxoid has been administered to volunteers for testing purposes and to occupationally at-risk workers. The schedule for the pentavalent toxoid calls for subcutaneous injections at 0, 2, and 12 weeks, followed by annual boosters. Recent advances in molecular cloning techniques and new knowledge about the molecular mechanisms of action of the toxins have opened up avenues for new botulinum vaccine development (Middlebrook, 1995).

Conclusions on the Health Effects of Botulinum Toxoid

Early studies of the initial univalent botulinum toxoids in the 1940s reported a significant number of local and systemic reactions (Middlebrook and Brown, 1995). Several studies that primarily focused on the efficacy of the botulinum toxoid vaccine (Fiock et al., 1962, 1963) noted moderate local or systemic reactions. Studies of the botulinum toxoid vaccine have not used active surveillance to systematically evaluate long-term health outcomes. This situation is unfortunately typical for all but a few vaccines.

The committee concludes that there is sufficient evidence of an association between botulinum toxoid vaccination and transient acute local and systemic effects (e.g., redness, swelling, fever) typically associated with vaccination.

The committee concludes that there is inadequate/insufficient evidence to determine whether an association does or does not exist between botulinum toxoid vaccination and long-term adverse health effects.

Multiple Vaccinations

Military personnel often receive several vaccinations as they prepare for service in an environment with many endemic diseases. People have expressed concerns that multiple vaccinations prior to and during Gulf War service may have caused adverse health effects.

Conclusions on the Health Effects of Multiple Vaccinations

Certain multiple vaccination regimens can lead to suboptimal antibody responses, but there is little evidence, largely because of a lack of active monitoring, of adverse clinical or laboratory consequences beyond the transient local and systemic effects seen frequently with any vaccination.

A group of 99 employees at Fort Detrick, Maryland, who received many vaccinations related to occupational requirements, were followed for up to 25 years to investigate the potential subclinical effects of intensive vaccination. The participants underwent physical examinations and laboratory testing in 1956, 1962, and 1971 (Peeler et al., 1958, 1965; White et al., 1974). No clinical sequelae attributable to intense long-term immunization could be identified in this cohort. None of the subjects suffered unexplained clinical symptoms requiring them to take sick leave that could be attributed to the vaccination program. There was some evidence of a chronic inflammatory response, as characterized by certain laboratory test abnormalities. However, these changes cannot necessarily be attributed to the vaccinations, because the workers studied were occupationally exposed to a number of virulent microbes. This series of longitudinal clinical studies had several shortcomings. However, the studies were valuable because careful monitoring did not disclose any evidence of serious unexplained illness in a cohort that received a series of intense vaccination protocols over many years.

Several studies of U.K. Gulf War veterans provide some limited evidence of an association between multiple vaccinations and long-term multisymptom outcomes, particularly for vaccinations given during deployment (Unwin et al., 1999; Hotopf et al., 2000). There are some limitations and confounding factors in these studies, and further research is needed.

The committee concludes that there is inadequate/insufficient evidence to determine whether an association does or does not exist between multiple vaccinations and long-term adverse health effects.

COMMENTS ON INCREASED RISK OF ADVERSE HEALTH OUTCOMES AMONG GULF WAR VETERANS

The committee reviewed the available scientific evidence in the peer-reviewed literature in order to draw conclusions about associations between the agents of interest and adverse health effects in all populations (see Table 1). The committee placed its conclusions in categories that reflect the strength of the evidence for an association between exposure to the agent and health outcomes. The committee could not measure the likelihood that Gulf War veterans' health problems are associated with or caused by these agents. To address this issue, the committee would need to compare the rates of health effects in Gulf War veterans exposed to the putative agents with the rates of those who were not exposed, which would require information about the agents to which individual veterans were exposed and their doses. However, as discussed throughout this report, there is a paucity of data regarding the actual agents and doses to which individual Gulf War veterans were exposed. Further, to answer questions about increased risk of illnesses in Gulf War veterans, it would also be important to know the degree to which any other differences between exposed and unexposed veterans could influence the rates of health outcomes. This information is also lacking for the Gulf War veteran population. Indeed most of the evidence that the committee used to form its conclusions about the association of the putative agents and health effects comes from studies of populations exposed to these agents in occupational and clinical settings, rather than from studies of Gulf War veterans. Due to the lack of exposure data on veterans, the committee could not extrapolate from the level of exposure in the studies that it reviewed to the level of exposure in Gulf War veterans. Thus, the committee could not determine the

TABLE 1 Summary of Findings

Sufficient Evidence of a Causal Relationship

Evidence is sufficient to conclude that a causal relationship exists between the exposure to a specific agent and a health outcome in humans. The evidence fulfills the criteria for sufficient evidence of an association (below) and satisfies several of the criteria used to assess causality: strength of association, dose–response relationship, consistency of association, temporal relationship, specificity of association, and biological plausibility.

- 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.

TABLE 1 *Continued*

Sufficient Evidence of an Association

Evidence is sufficient to conclude that there is a positive association. That is, a positive association has been observed between an exposure to a specific agent and a health outcome in human studies in which chance, bias, and confounding could be ruled out with reasonable confidence.

- Pyridostigmine bromide and transient acute cholinergic effects in doses normally used in treatment and for diagnostic purposes.
- Anthrax vaccination and transient acute local and systemic effects.
- Botulinum toxoid vaccination and transient acute local and systemic effects.

Limited/Suggestive Evidence of an Association

Evidence is suggestive of an association between exposure to a specific agent and a health outcome in humans, but is limited because chance, bias, and confounding could not be ruled out with confidence.

- Exposure to sarin at doses sufficient to cause acute cholinergic signs and symptoms and subsequent long-term health effects.

Inadequate/Insufficient Evidence to Determine Whether an Association Does or Does Not Exist

The available studies are of insufficient quality, consistency, or statistical power to permit a conclusion regarding the presence or absence of an association between an exposure to a specific agent and a health outcome in humans.

- Exposure to uranium and lung cancer at higher levels of cumulative exposure (>200 mSv or 25 cGy).
- Exposure to uranium and lymphatic cancer; bone cancer; nervous system disease; nonmalignant respiratory disease; or other health outcomes (gastrointestinal disease, immune-mediated disease, effects on hematological parameters, reproductive or developmental dysfunction, genotoxic effects, cardiovascular effects, hepatic disease, dermal effects, ocular effects, or musculoskeletal effects).
- Pyridostigmine bromide and long-term adverse health effects.
- Exposure to sarin at low doses insufficient to cause acute cholinergic signs and symptoms and subsequent long-term adverse health effects.
- Anthrax vaccination and long-term adverse health effects.
- Botulinum toxoid vaccination and long-term adverse health effects.
- Multiple vaccinations and long-term adverse health effects.

Limited/Suggestive Evidence of No Association

There are several adequate studies covering the full range of levels of exposure that humans are known to encounter, that are mutually consistent in not showing a positive association between exposure to a specific agent and a health outcome at any level of exposure. A conclusion of no association is inevitably limited to the conditions, levels of exposure, and length of observation covered by the available studies. In addition, the possibility of a very small elevation in risk at the levels of exposure studied can never be excluded.

- Exposure to uranium and lung cancer at cumulative internal dose levels lower than 200 mSv or 25 cGy.
- Exposure to uranium and clinically significant renal dysfunction.

likelihood of increased risk of adverse health outcomes among Gulf War veterans due to exposure to the agents examined in this report.

RESEARCH RECOMMENDATIONS

The committee's charge was to review the scientific literature on the potential health effects of agents to which Gulf War veterans may have been exposed. Of the many stressors and biological and chemical agents in the Gulf War theater, this report has reviewed the literature on the agents that were of most concern to the veterans and their representatives. Subsequent IOM studies will examine the literature on other Gulf War-related agents.

The committee considered the evidence for each of the agents in turn, as if each one were the only risk factor for adverse health effects. It did so because committee members sought to learn how each agent, in the absence of all of the others, might affect human health. The committee realized through the course of this study, however, that there may also be a need to examine the impact of the total experience of deployment and war on veterans' health. Such an approach may help elucidate the nature of the illnesses in Gulf War veterans in a way that is not possible by examining single agents. Unfortunately, most of the studies conducted to date focus only on single agents. Yet integrating the various stressors, biological and chemical exposures, the complexities faced by military personnel during all phases of deployment, and the issues surrounding war may provide a more realistic approach toward understanding veterans' health issues and may provide insights for preventing illnesses in future deployments.

The committee has developed the recommendations in Table 2 for future research, based on its review of the literature on each of the putative agents. These recommendations highlight areas of scientific uncertainty and, if implemented, will help to resolve important questions about the effect of the Gulf War on the health of the veterans.

Finally, this report takes its place alongside several other recent IOM reports on the health of Gulf War veterans. Although the conclusions and recommendations presented here will not end the controversy surrounding Gulf War veterans' illnesses, this report will provide a scientific basis for consideration by the Department of Veterans Affairs as they develop a compensation program for veterans. The committee hopes that its deliberations, along with the work of many others, will add to the body of accumulating knowledge about the health of Gulf War veterans.

TABLE 2 Research Recommendations

Biological, Chemical, and Psychological Interactions

- Research on the interactions among the multiple agents and stressors to which military personnel were exposed as a result of the Gulf War conflict.

Depleted Uranium

- Continued follow-up of the Baltimore cohort of Gulf War veterans with DU exposure. Long-term studies of the health of other Gulf War veterans at high risk for DU exposure (e.g., cleanup or radiation control units).
- Continued follow-up of the cohorts of uranium processing workers.
- Additional studies of the effects of DU in animals.

Sarin

- Long-term follow-up of populations exposed to sarin in the Matsumoto and Tokyo terrorist attacks.
- Studies in experimental animals to investigate the long-term effects of an acute, short-term exposure to sarin at doses that do not cause overt cholinergic effects and minimal acetylcholinesterase inhibition.
- Research on genetic factors that may alter susceptibility to sarin toxicity.

Pyridostigmine Bromide

- Research on chemical interactions between PB and other agents such as stress, and certain insecticides.
- Research on genetic factors (e.g., genetic polymorphisms of butyrylcholinesterase, paraoxonase) that may alter susceptibility to the effects of PB.
- Epidemiologic studies on the possible long-term health effects of PB.

Vaccines

- Long-term longitudinal studies of participants in the Anthrax Vaccine Immunization Program that would actively monitor and systematically collect and analyze data about symptoms, functional status, and disease status.
- Long-term systematic research to examine potential adverse effects of anthrax and botulinum toxoid vaccination in multiple species and strains of animals.
- Careful study of current symptoms, functional status, and disease status in cohorts of Gulf War veterans and Gulf War-era veterans for whom vaccination records exist.

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CONTENTS

1

Introduction

On August 2, 1990, Iraqi armed forces invaded Kuwait; within 5 days the United States began to deploy troops to Southwest Asia (SWA)[1] in Operation Desert Shield. Intense air attacks against the Iraqi armed forces began on January 16, 1991, and opened a phase of the conflict known as Operation Desert Storm. Oil-well fires became visible by satellite images as early as February 9, 1991; the ground war began on February 24, and by February 28, 1991, the war was over. The oil fires were extinguished by November 1991. The last troops to participate in the ground war returned home on June 13, 1991. In all, approximately 697,000 U.S. troops had been deployed to the Persian Gulf area during the conflict.

Although considered an extraordinarily successful military operation with few battle casualties and deaths, veterans soon began reporting numerous health problems that they attributed to their participation in the Gulf War. Although the majority of men and women who served in the Gulf War returned to normal activities, a large number of veterans have had a range of unexplained illnesses including chronic fatigue, muscle and joint pain, loss of concentration, forgetfulness, headache, and rash (Chapter 2).

The men and women who served in the Persian Gulf region were potentially exposed to a wide range of biological and chemical agents including sand, smoke from oil-well fires, paints, solvents, insecticides, petroleum fuels and their combustion products, organophosphate nerve agents, pyridostigmine bromide (PB), depleted uranium (DU), anthrax and botulinum toxoid vaccinations,

[1]The Southwest Asia theater of operations included the Persian Gulf, Iraq, Kuwait, Saudi Arabia, the Red Sea, Gulf of Oman, Gulf of Aden, portions of the Arabian Sea, Oman, Bahrain, Qatar, and the United Arab Emirates.

and infectious diseases, in addition to psychological and physiological stress. Veterans have become increasingly concerned that their ill health may be related to exposure to these agents and circumstances.

In response to the veterans' concerns, the Department of Veterans Affairs (VA) approached the National Academy of Sciences and requested that the Institute of Medicine (IOM) conduct a study to extensively review the literature and summarize the strength of the scientific evidence concerning the association between health effects and the chemical and biological compounds that were likely present during the Gulf War.

To carry out the charge as requested by the VA, the IOM formed the Committee on Health Effects Associated with Exposures During the Gulf War. As the committee began its deliberations in January 1999, one of its first tasks was to determine the initial group of compounds for study. The committee decided that the compounds of most concern to the veterans should be selected for initial review. Following meetings with leaders of different veterans' organizations, the committee decided it would begin this first phase by studying the following compounds: depleted uranium, chemical warfare agents (sarin and cyclosarin), pyridostigmine bromide, and vaccines (anthrax and botulinum toxoid). Subsequent studies will examine the remaining agents.

Subsequent to the VA–IOM contract, two public laws were passed: the Veterans Programs Enhancement Act of 1998 (Public Law 105-368) and the Persian Gulf War Veterans Act of 1998 (Public Law 105-277). Each law mandated studies similar to the study already agreed upon by the VA and IOM. These laws detail several comprehensive studies on veterans' health and specify numerous biological and chemical hazards that may potentially be associated with the health of Gulf War veterans.

It should be noted that the charge to the IOM committee was not to determine whether a unique Gulf War syndrome exists, nor was it to make judgments regarding levels of exposure of veterans to the putative agents. Additionally, the committee's charge was not to focus on broader issues, such as the potential costs of compensation for veterans or policy regarding such compensation. These decisions remain the responsibility of the Secretary of Veterans Affairs (VA). 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 these health effects as the VA develops a compensation program for Gulf War veterans.

ADDRESSING GULF WAR HEALTH ISSUES

Past and Current Efforts

In the years since the Gulf War, a number of federal and private sector efforts have explored the causes of and treatments for the illnesses of Gulf War veterans. Initial efforts focused on concerns about potential health effects of the Kuwait oil-well fires. Subsequently, concern has broadened to encompass possi-

ble health effects of other agents and treatments for the veterans' health problems. Extensive research and policy efforts continue. In addition to the panels identified in Box 1.1, there are ongoing efforts by individual veterans, veteran service organizations, academia, Congress, federal agencies, private sector organizations, and others. These efforts focus on the spectrum of work needed to fulfill the goal of improving the health of Gulf War veterans who are ill and preventing illnesses in future deployments. This work includes clinical research on the effectiveness of potential treatments, improving exposure models, epidemiologic research on the health status of Gulf War veterans, research on the nature of the veterans' illnesses, and studies on the potential adverse health effects of the agents that were likely present in the Gulf War.

Complexities in Resolving Gulf War Health Issues

Investigations of the health effects of past wars have often focused on narrowly defined hazards or health outcomes, such as infectious diseases (e.g., typhoid, malaria) during the Civil War, specific chemical hazards (e.g., mustard gas in World War I, Agent Orange and other herbicides in Vietnam), and combat injuries. A discussion of the possible health effects of the Gulf War, however, involves many complex issues, some of which are explored below. These include exposure to multiple biological and chemical agents, limited exposure information, individual variability factors, and illnesses that are often nonspecific and lack defined medical diagnoses or treatment protocols. While, the committee was not tasked with addressing these issues it presents them in this introductory chapter to acknowledge the difficulties faced by veterans, researchers, policymakers, and others in reaching an understanding about the veterans' ill health.

Multiple Exposures and Chemical Interactions

Although Operation Desert Shield/Desert Storm was relatively short in duration, military personnel were potentially exposed to numerous agents. Many of the exposures are not unique to the Gulf War, however, the number of agents and the combination of agents to which the veterans may have been exposed make it difficult to determine whether any one agent, or combination of agents, is the cause of Gulf War veterans' illnesses. These include preventive measures (e.g., PB, vaccines, pesticides, insecticides), hazards of the natural environment (e.g., sand, endemic diseases), job-specific exposures (e.g., paints, solvents, diesel fumes), war-related exposures (e.g., smoke from burning oil-well fires, DU), and hazards from cleanup operations (e.g., sarin and cyclosarin). Thus, military personnel may have been exposed to a variety of agents, at varying doses, and lengths of time. The literature on the agents, however, is quite limited with regard to combinations of biological and chemical agents and their interactions.

BOX 1.1
SELECTED PAST AND ONGOING COMMITTEES AND PANELS ADDRESSING GULF WAR HEALTH CONCERNS

1992
- Expert Panel on Petroleum Toxicity

1993
- Office of Technology Assessment Workshop on Persian Gulf Health
- Defense Science Board Task Force on Persian Gulf War Health Effects (1993–1994)
- IOM Committee to Review the Health Consequences of Service During the Persian Gulf War (1993–1996)

1994
- National Institutes of Health Technology Assessment Workshop—Persian Gulf Experience and Health
- Persian Gulf Veterans Coordinating Board (1994–present)
- Persian Gulf Expert Scientific Panel (1994–1999)
- IOM Committee to Review the Department of Defense's (DoD's) Comprehensive Clinical Evaluation Program (1994–1998 in two phases)

1995
- Persian Gulf Investigation Team and Declassification Program
- Task Force on Analysis and Declassification of Intelligence Records (Central Intelligence Agency)
- Presidential Advisory Committee on Gulf War Veterans' Illnesses (1995–1997)

1996
- Office of the Special Assistant for Gulf War Illnesses (1996–present)

1997
- IOM Committee on the Evaluation of the VA Uniform Case Assessment Protocol (1997–1998)
- Special Investigative Unit on Gulf War Illnesses (1997–1998)

1998
- Presidential Special Oversight Board for DoD Investigations of Gulf War Chemical and Biological Incidents (1998–present)
- IOM Committee on Measuring the Health of Gulf War Veterans (1998–1999)

1999
- IOM Committee on Health Effects Associated with Exposures During the Gulf War (1999–2000)
- IOM Committee on Identifying Effective Treatments for Gulf War Veterans' Health Problems (1999–present)
- Military and Veterans Health Coordinating Board (1999–present)

Limitations of Exposure Information

Determining whether or not Gulf War veterans face an increased risk of illness because of their exposures during the Gulf War requires extensive information about each exposure (e.g., the actual agent(s), duration of exposure, route of entry, internal dose) and documentation of adverse reactions. Unfortunately, very little is known about most Gulf War exposures. After the ground war, an environmental monitoring effort was initiated primarily because of concerns related to smoke from oil-well fires rather than for the other agents to which the troops may have been exposed. Consequently, exposure data for other agents are lacking or are limited.

While a variety of exposure assessment tools are being used in ongoing research to fill gaps in exposure information there are limitations to accurate reconstruction of past exposure events. For example, surveys of veterans are used to obtain recollections about agents to which they may have been exposed, although survey results may be limited by recall bias (see Chapter 3). Models are being refined to estimate exposures to sarin and cyclosarin, however, it is difficult to accurately incorporate intelligence information, meteorological data, transport and dispersion data and troop unit location information. Extensive efforts are under way to model and obtain information on potential exposures to depleted uranium, smoke from oil-well fires, and other agents. Although modeling efforts are important for discerning the details of the exposures of Gulf War veterans, these efforts are not yet complete and will require external review and validation. Further, even if there were accurate troop locations, the location of individual soldiers would be very uncertain. Because of the limitations in the exposure data, it is difficult to determine the likelihood of increased risk for disease or other adverse health effects in Gulf War veterans.

Individual Variability

Differences among individuals in their genetic, biological, psychological, and social vulnerabilities add to the complexities in determining health outcomes related to specific agents. Sensitive individuals will exhibit different responses to the same agents than members of the population without the susceptibility. For example, an individual may be a poor metabolizer of a particular substance, depending on his or her genetic makeup. Such an individual may be at higher or lower risk for specific health effects due to exposure to certain agents. Researchers are investigating the genotypes coding for two forms of an enzyme that differ in the rate at which they hydrolyze certain organophosphates (including sarin). Lower hydrolyzing activity would mean that despite identical exposure to sarin, more sarin would be bioavailable in those individuals resulting in increased anticholinesterase effects (see Chapter 5).

Unexplained Symptoms

Many Gulf War veterans suffer from an array of health problems and symptoms (e.g., fatigue, muscle and joint pain, memory loss, rash) that are not specific to any one disease and are not easily classified by standard diagnostic coding systems. Population-based studies have found higher prevalence of self-reported symptoms in Gulf War veterans compared to nondeployed Gulf War era veterans or other control groups (see Chapter 2; Iowa Persian Gulf Study Group, 1997; Goss Gilroy, 1998; Unwin et al., 1999). All Gulf War veterans do not experience the same array of symptoms, which has complicated ongoing efforts to determine if there is a unique Gulf War syndrome or if there is overlap with other symptom-based disorders. Thus, the nature of the symptoms suffered by many Gulf War veterans does not point to an obvious diagnosis, etiology, or standard treatment (see Chapter 2).

THE GULF WAR SETTING

Although the committee's charge was to review the scientific evidence on the possible health effects of various agents to which Gulf War veterans were exposed, the committee realized at the onset that it needed to have as complete an understanding of the Gulf War experience as possible. The committee sought to understand the Gulf War setting and veterans' experiences. For that reason, the committee met with representatives of veterans' groups and opened its meetings whenever possible to hear from veterans, researchers, and other members of the interested public (see Appendixes A and B).

The following information provides a context for the many scientific articles that the committee reviewed and provides an appreciation (albeit limited) of the collective experiences of Gulf War veterans. This information is compiled from many sources including presentations by veterans and other speakers at the committee's public meetings (see Appendix B) (Gunby, 1991; NIH, 1994; Hyams et al., 1995; IOM, 1995, 1996, 1999; Persian Gulf Veterans Coordinating Board, 1995; Ursano and Norwood, 1996; PAC, 1996, 1997; Lawler et al., 1997; Joellenbeck et al., 1998; U.S. Department of Veterans Affairs, 1998).

Deployment

The pace of the buildup for the war was unprecedented. Within 5 days after Iraq invaded Kuwait, the United States and other coalition countries began moving troops into the region. By September 15, 1990, the number of American service members reached 150,000 and included nearly 50,000 reservists. Within the next month, another 60,000 troops arrived in Southwest Asia, and in November an additional 135,000 reservists and guard members were called up. By February 24, 1991, more than 500,000 U.S. troops had been deployed to the Persian Gulf region.

The Gulf War reflected many changes from previous wars in the demographic composition of military personnel and uncertain conditions for many reservists. Of the nearly 700,000 U.S. troops who fought in Operation Desert Shield/Desert Storm, almost 7 percent were women and about 17 percent were from National Guard and reserve units. Additionally, military personnel were, overall, older than those who had participated in previous wars. Rapid mobilization exerted substantial pressures on those who were deployed, disrupting lives, separating families, and for reserve and guard units, creating uncertainty about whether jobs would be available when they returned to civilian life.

Living Conditions

Combat troops were crowded together in warehouses and tents upon arrival and then often moved to isolated desert locations. Most troops lived in tents and slept on cots lined up side by side, affording virtually no privacy or quiet. Sanitation was often primitive, with strains on latrines and communal washing facilities; feminine hygiene products were initially in short supply. Hot showers were infrequent, the time interval between laundering of uniforms was sometimes long, and desert flies were a constant nuisance, as were scorpions and snakes. Additionally, military personnel worked long hours and had restricted outlets for relaxation. Troops were ordered not to fraternize with local people, and alcohol was prohibited in deference to religious beliefs in the host countries. A mild, traveler's type of diarrhea affected more than 50 percent of the troops in some units. Fresh fruits and vegetables from neighboring countries were identified as the risk factor and were removed from the diet. Thereafter, the diet consisted mostly of prepackaged foods and bottled water.

For the first two months of troop deployment (August and September) the weather was extremely hot and humid, with air temperatures as high as 115°F and temperatures of the sand reaching 150°F. Except for coastal regions, the relative humidity was less than 40 percent. Troops had to drink large quantities of water to prevent dehydration. While the summers were hot and dry, temperatures in winter (December through March) were cold, with wind chill temperatures at night dropping well below freezing. Wind and blowing sand made protection of skin and eyes imperative. Individuals were not allowed to wear contact lenses, except in air-conditioned areas that were protected from sand. Goggles and sunglasses helped somewhat, but visibility was often poor.

Environmental and Chemical Exposures

Certainly the most visually dramatic environmental event of the Gulf War was the smoke from more than 750 oil-well fires. Smoke plumes rose and combined to form giant plumes that could be seen for hundreds of kilometers. In addition to oil-well fires, there were other potential sources of exposure to petroleum-based products. Kerosene, diesel, and leaded gasoline were used in un-

vented tent heaters, cooking stoves, and portable generators. Petroleum products, including diesel fuels, were used to suppress sand and dust. Additionally, petroleum fuels were used for burning waste and trash.

Pesticides, including dog flea collars, were widely used by troops in the Gulf to combat the region's ubiquitous insect and rodent populations. Pesticides used included methyl carbamates, organophosphates, pyrethroids, and chlorinated hydrocarbons. Although guidelines for use were strict, there were many reports of misuse.

There were many possible exposures related to particular occupational activities in the Gulf War. The majority of occupational chemical exposures appear to have been related to repair and maintenance activities including battery repair (corrosive liquids), cleaning or degreasing (solvents, including chlorinated hydrocarbons), sandblasting (abrasive particulates), vehicle repair (asbestos, carbon monoxide, organic solvents), weapon repair (lead particulates), and welding or cutting (chromates, nitrogen dioxide, heated metal fumes). Additionally, troops painted vehicles and equipment used in the Gulf with chemical agent-resistant coating (CARC) either before being shipped to the Gulf or at ports in Saudi Arabia. Because working conditions in the field were not ideal, recommended occupational hygiene standards may not have been followed at all times.

Exposure of U.S. personnel to depleted uranium occurred as the result of friendly fire incidents, cleanup operations, and accidents (including fires). Others may have inhaled DU dust through contact with DU-contaminated tanks or munitions (see Chapter 4).

Threat of Chemical and Biological Warfare

When U.S. troops first arrived in the Gulf, they had no way of knowing if they would be exposed to biological and chemical weapons. Iraq had used such weapons in fighting Iran and in attacks on the Kurdish minority in Iraq. Military leaders feared that the use of such weapons in the Gulf could result in the deaths of tens of thousands of Americans. Therefore, in addition to the standard vaccinations given prior to military deployment, about 150,000 troops received anthrax vaccine and about 8,000 received botulinum toxoid vaccine (see Chapter 7). Additionally, troops were given blister packs of 21 tablets of pyridostigmine bromide to protect against possible chemical warfare. Troops were to take PB upon the orders of a commanding officer when chemical warfare attack was believed to be imminent (see Chapter 6).

Chemical sensors and alarms were distributed throughout the region to warn of such attacks. The alarms were extremely sensitive and could be triggered by many substances including some organic solvents, vehicle exhaust fumes, and insecticides. Although follow-up analysis by the Department of Defense (DoD) found no evidence of the use of chemical warfare agents, the alarms sounded frequently, and troops responded by donning the confining protective gear and ingesting PB as an antidote to the effects of nerve gas. In addition to the alarms, there were widespread reports of dead sheep, goats, and camels, which troops

were taught could be an indication of the use of chemical or biological weapons. The sounding of these alarms, and the reports of dead animals, plus rumors that other units had been hit by chemical warfare agents, caused the troops to be concerned that they would be or had been exposed to these agents.

Despite the small numbers of U.S. personnel injured or killed during combat in the Gulf War, the troops, as in any war, faced the fear of death, injury, or capture by the enemy. They witnessed the many horrors of war, including dead bodies.

After the war, there was the potential for other exposures, including U.S. demolition of a munitions storage complex at Khamisiyah, Iraq, which—unbeknownst to demolition troops at the time—contained stores of sarin and cyclosarin (see Chapter 5).

It has been documented from the Civil War to the Gulf War that a variety of physical and psychological stressors placed military personnel at high risk for adverse health effects (Hyams et al., 1996). In addition to the threat or experience of combat, the Gulf War involved rapid and unexpected deployment, harsh living conditions, continuous anticipation of exposure to chemical and biological agents, environmental pollution from burning oil fires, and family disruption and financial strain.

SCOPE OF THE REPORT

The committee was charged with conducting a review of the scientific literature on the possible health effects of agents to which Gulf War veterans may have been exposed. The breadth of this review included all relevant toxicological, animal, and human studies. Because only a few studies related directly to veterans' exposures, the committee reviewed studies of any human populations—including veterans—that had been exposed to the agents of concern at any dose. These studies come primarily from occupational, clinical, and healthy volunteer settings.

By examining the full range of evidence for health outcomes in different populations, the committee addressed the question, *Could exposure to a given agent be associated with a specific health outcome?* As discussed in Chapter 3, an association between a specific agent and a health outcome does not mean that exposure to the agent invariably results in the health outcome or that all cases of the health outcome are related to exposure to the specific agent. Such complete correspondence between exposure and disease is the exception in the study of disease in large populations (IOM, 1994).

The committee began its task by hearing from many veterans' organizations, because the committee realized that it could not conduct a credible scientific review without an understanding of veterans' experiences and perspectives. Thus, to supplement the scientific process, the committee opened several of its meetings to veterans and other interested individuals. The committee held a scientific workshop (see Appendix A) and two public meetings (Appendix B). They also received information in written form from veterans' organizations,

veterans, and other interested persons who made the committee aware of their experiences or their health status and provided information about research. This information helped the committee by providing details on the Gulf War experience, in identifying particular agents and health issues of concern, and in providing a context for the committee's work.

The committee and staff reviewed more than 10,000 abstracts of scientific and medical articles related to the agents selected for study. The full text of more than 1,000 peer-reviewed journal articles, many of which are described in this report, were carefully reviewed by the committee (see Appendix C for a complete description of the committee's literature review strategy).

ORGANIZATION OF THE REPORT

Chapter 2 provides an overview of major studies that have been conducted on the health of Gulf War veterans. It highlights the complexity of efforts to understand the nature of the veterans' illnesses, reviews some of the many studies that have provided data on the symptomatology of illnesses in Gulf War veterans, and discusses the limitations of these studies. Chapter 3 outlines the methods used by the committee to review the literature, the issues it debated while considering the evidence, and its criteria for reaching conclusions about the strength of the evidence for or against associations between adverse health effects and specific agents.

The next four chapters review the scientific literature on each of the agents chosen for study: depleted uranium (Chapter 4), sarin and cyclosarin (Chapter 5), pyridostigmine bromide (Chapter 6), and the anthrax and botulinum toxoid vaccines (Chapter 7). Each of these chapters explains the use of the agent during the Gulf War, contains an overview of the toxicology of the agent, describes the results of animal studies, and provides detailed descriptions of human studies. Further, when evidence was available on combinations of chemicals or other agents, the committee includes that information in its discussion. The committee provides conclusions in each of the chapters about the strength of the relationship between the agent and the possibility of adverse health outcomes. Finally, where there are gaps in the information, the committee makes recommendations for future research efforts in those areas (Chapter 8).

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CONTENTS

2

Illnesses in Gulf War Veterans

Almost a decade after the Gulf War, questions persist about illnesses reported by veterans. A significant number of veterans report having fatigue, skin rash, headache, muscle and joint pain, and loss of memory (Joseph, 1997; Murphy et al., 1999). An increased prevalence of these symptoms has been borne out by large controlled studies of deployed compared to nondeployed military personnel[1] from three countries—the United States, the United Kingdom, and Canada. That so many Gulf War veterans have unexplained[2] symptoms has prompted concerns about their exposure to potentially hazardous agents during the Gulf War. The U.S. government has made a substantial investment in health research to understand veterans' illnesses, search for their cause(s), and find effective treatments (CDC, 1999; Research Working Group, 1999).

This chapter describes the research that has addressed three fundamental questions about illnesses in Gulf War veterans:[3] (1) what is the nature and prevalence of veterans' symptoms and illnesses; (2) do their unexplained sym-

[1]Many studies have compared the health of military personnel deployed to the Gulf War with military personnel who were not deployed to the Gulf War but served during the same period (Gulf War era). Some studies have a comparison cohort of military personnel who served in another deployment (e.g., Bosnia).

[2]The terms "unexplained symptoms" or "unexplained illnesses" means that health complaints cannot be accounted for, or explained by, current medical diagnoses.

[3]This chapter employs the term "Gulf War veterans" in the broadest sense. Unless otherwise specified, the term denotes all military personnel who served in the Gulf War theater between August 2, 1990, and June 13, 1991, regardless of whether they later continued on active duty, returned to the reserves or National Guard, or left military service.

toms warrant classification as a new syndrome; and (3) are exposures to specific biological and chemical agents during the Gulf War associated with veterans' symptoms and illnesses?

This chapter's exclusive focus is on health studies of Gulf War veterans. The questions posed above are designed to guide the reader through a complex body of research. The chapter summarizes studies of veterans' mortality, hospitalizations, and diagnosable illnesses and provides a brief overview of the Gulf War veterans' registry programs established by the Department of Veterans Affairs (VA) and the Department of Defense (DoD). The chapter also examines in greater depth the epidemiologic studies that have been conducted to date—on general health status and on specific health endpoints. The information presented here provides background for the reader and the context for committee members as they considered evidence related to health effects of the agents selected for study. Later chapters deal with the specific agents and their health effects in any population, including veterans.

REGISTRY PROGRAMS

Approximately 697,000 U.S. service men and women were deployed in Operations Desert Shield/Desert Storm in 1990 and 1991 (PAC, 1996). The demographic composition of this deployment was more diverse than in past deployments; there were greater racial and ethnic diversity, more women, and more reserves and National Guard troops (Table 2.1).

Soon after the war ended in 1991, veterans began to seek medical treatment for a variety of symptoms and illnesses (PAC, 1996). The Department of Defense and the Department of Veterans Affairs responded to veterans' health concerns by establishing programs for veterans to voluntarily receive clinical examinations largely for diagnostic purposes. By 1994, these registry programs had been revised and renamed the Comprehensive Clinical Evaluation Program (hereinafter called the DoD registry) and the Persian Gulf Registry and Uniform Case Assessment Protocol (hereinafter called the VA registry), respectively. The programs are similarly structured: they begin with an initial physical examination, including patient and exposure history and screening laboratory tests, followed by the opportunity for referral to more specialized testing and consultation if needed (Joseph, 1997; Murphy et al., 1999).[4] About 125,000 Gulf War veterans underwent registry health examinations through March 1999 (IOM, 1999a), the majority conducted under the auspices of the VA. These programs continue to register participants.

The most common symptoms reported between 1992 and 1997 from among 52,835 participants of the VA registry were fatigue, skin rash, headache, muscle and joint pain, and loss of memory (Table 2.2) (Murphy et al., 1999). An almost

[4]Several independent advisory committees have reviewed these programs and made recommendations for their refinement (NIH, 1994; IOM, 1995–1998; PAC, 1996).

TABLE 2.1 Demographic Characteristics of U.S. Gulf War Troops

Characteristic	Percentage of Troops[a]
Gender	
Male	93
Female	7
Age (mean) in 1991 (years)	27
Race or ethnicity	
White	70
African American	23
Hispanic	5
Other	2
Rank	
Enlisted	90
Officer	10
Military branch	
Army	50
Navy	23
Marines	15
Air Force	12
Military status	
Active duty	83
Reserves or National Guard	17

[a]There were approximately 697,000 U.S. military personnel.

SOURCE: Joseph, 1997.

identical set of symptoms was reported most frequently among the approximately 20,000 participants in the DoD registry (CDC, 1999). Veterans classified in the DoD registry as having "signs, symptoms, and ill-defined conditions" most frequently complained of fatigue, headache, and memory loss (Roy et al., 1998). Clinicians were able to arrive at a primary diagnosis for about 82 percent of symptomatic DoD registry participants (Joseph, 1997) and for a similar fraction of VA registry participants (Murphy et al., 1999) (Table 2.2). A registry program established by the United Kingdom Ministry of Defence for U.K. Gulf War veterans found similar types and frequencies of symptoms and diagnoses (Coker et al., 1999). Across the registries, musculoskeletal diseases; mental disorders; and symptoms, signs, and ill-defined conditions[5] were the three most

[5]"Symptoms, signs, and ill-defined conditions" refers to International Classification of Diseases, Ninth Revision, Classical Modification (ICD-9-CM) codes 780–799, which are reserved for 160 subclassifications of ill-defined, common conditions not

common diagnostic categories, together accounting for more than 50 percent of primary diagnoses (CDC, 1999).

Registry programs provided an early glimpse into veterans' symptoms and the difficulties of fitting symptoms into standard diagnoses. As self-selected case series of veterans who presented for care, registries cannot, and were not intended to, be representative of the symptoms and illnesses of the entire group of Gulf War veterans. Nor were registries designed with control groups or with diagnostic standardization across the multiple sites at which examinations took place (Joseph, 1997; Roy et al., 1998). Finally, owing to their reliance on stan-

TABLE 2.2 Most Frequent Symptoms and Diagnoses Among 53,835 Participants in the VA Registry (1992–1997)

Symptoms or Diagnoses	Percentage
Self-Reported Symptoms	
Fatigue	20.5
Skin rash	18.4
Headache	18.0
Muscle, joint pain	16.8
Loss of memory	14.0
Shortness of breath	7.9
Sleep disturbances	5.9
Diarrhea and other gastrointestinal symptoms	4.6
Other symptoms involving skin	3.6
Chest pain	3.5
No complaint	12.3
Diagnosis (ICD-9-CM)	
No medical diagnosis	26.8
Musculoskeletal and connective tissue	25.4
Mental disorders	14.7
Respiratory system	14.0
Skin and subcutaneous tissue	13.4
Digestive system	11.1
Nervous system	8.0
Infectious diseases	7.1
Circulatory system	6.4
Injury and poisoning	5.3
Genitourinary system	3.0
Neoplasm	0.4

SOURCE: Murphy et al., 1999.

coded elsewhere in ICD-9-CM or without a distinct physiological or psychological basis (U.S. DHHS, 1998).

dard diagnostic classifications, registries were not designed to probe for novel diagnoses[6] or to search for biological correlates. Thus, because of their methodological limitations, registry studies cannot stand alone as a basis for conclusions or for the conduct of research.

Registry programs are, however, a valuable resource for information and for generating hypotheses. These hypotheses can be tested in more rigorous epidemiologic studies with control groups in order to estimate the population prevalence of symptoms among Gulf War veterans and to compare these to rates among otherwise similar troops who were not deployed to the Gulf War.

EPIDEMIOLOGIC STUDIES OF VETERANS' SYMPTOMS AND GENERAL HEALTH STATUS

A number of epidemiologic studies have been conducted on the health status of Gulf War veterans. The driving issues behind many of these studies are to determine (1) the nature of symptoms and symptom clusters; (2) whether symptom clusters constitute a new, unique syndrome; and/or (3) what types of exposures may have produced the symptoms.

The second issue highlighted above—the quest to define a new syndrome—requires some explanation. The question is whether or not these unexplained symptoms constitute a syndrome(s) and, if so, are they best studied and treated as a unique new syndrome(s) or a variant form(s) of an existing syndrome (see Appendix D). The finding of any new set of unexplained symptoms in a group of patients does not automatically qualify as a new syndrome.[7] It represents the beginning of a process involving many types of studies to demonstrate that the patients are affected by a unique clinical entity distinct from all other established clinical diagnoses.

The process of defining a new syndrome usually begins with a case definition that lists classification criteria to distinguish the potentially new patient population from patients with existing clinical diagnoses. Development of the first case definition is a vital milestone designed to spur research and surveillance. More like a hypothesis than a conclusion, the first case definition is an early step in the process and is often revised as more evidence comes to light. Case definitions usually are a mix of clinical, demographic, and/or laboratory criteria. Clinical criteria refer to signs (physical examination findings) and symptoms (subjective experiences or reports of patients). Demographic criteria refer to age, gender, ethnicity, or other individual characteristics or exposure-related variables. Laboratory criteria are biological measures of either pathology or etiology (e.g., x-ray, blood test).

[6]Registries rely on the ICD-9-CM (Joseph, 1997; Murphy et al., 1999).

[7]A syndrome is a unique set or cluster of symptoms, signs, and/or laboratory tests without known pathology or etiology (Scadding, 1996).

One method of developing an operational case definition is a statistical technique known as factor analysis (Ismail et al., 1999). Factor analysis is useful in identifying a small number of correlated variables from among a much larger number of observed variables, such as the symptoms that are reported in a survey of veterans. Factor analysis aggregates survey responses into statistical groupings of factors that may or may not have biological plausibility or clinical relevance. Several researchers have used factor analysis in their studies (described later in this chapter) on the health of Gulf War veterans. When factor analysis is employed in studies of veterans, the observed variables are measurements of veterans' symptoms, and the fundamental factors are symptom groupings that may represent a potentially new syndrome. Any new syndrome (defined by factor analysis or other means) may have a distinct, albeit often unknown, etiology and pathogenesis (Taub et al., 1995). It is recognized that factor analysis has the potential to generate syndromes that may not be reproduced when a new population is examined.

When evidence is presented that the case definition—defined by factor analysis or other methods—successfully singles out a new patient population from comparison groups, the case definition may gain recognition by the medical establishment as a new syndrome (see Appendix D). There are many advantages to defining and classifying a new syndrome. The foremost advantage is to create a more homogeneous patient population, a crucial step for determining prevalence and ushering in diagnosis and treatment. A potential disadvantage is the mislabeling or misclassification of a condition, which can thwart progress for years, if not decades (Aronowitz, 1991). Classification of a new patient population also stimulates further understanding of the natural history of the disease, risk factors, and ultimately, etiology and pathogenesis. As more knowledge unfolds about etiology and pathogenesis, the classification of an established syndrome can rise to the level of a disease. The renaming of a syndrome as a disease[8] implies that the etiology or pathology has been identified.

Population-Based Studies

This section summarizes findings of population-based studies of Gulf War veterans. The next section summarizes findings from studies using other types of epidemiological designs. 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 (e.g., a particular base, a particular branch such as the Air Force). Studies of military units or other military

[8]The term "disease" is defined as an abnormality in body structure or function with known etiology (e.g., virus, abnormal gene, toxin) and/or pathology (detectable lesion).

subgroups are less representative of the broader Gulf War veteran population than are population-based studies.

Large population-based studies of Gulf War veterans have been conducted in each of the three major countries participating in the Gulf War coalition (e.g., the United States, Canada, and the United Kingdom). These studies have shown consistent findings, in both the nature of unexplained symptoms and their deleterious impact on functioning. Summary features of these studies appear in Table 2.3, along with those of other epidemiologic studies.

Virtually all epidemiologic studies of Gulf War veterans, regardless of study design, rely on self-reports of both symptoms and exposures. As discussed in Chapter 3, studies based on self-reports have inherent limitations because of potential inaccuracies in recalling past events and difficulty in verifying the reports. Most of the larger epidemiologic studies described here were conducted through mail or telephone surveys, precluding the possibility of clinical examination and diagnosis. Comparison groups were veterans of the same era who were not deployed to the Gulf War. More comprehensive reviews of epidemiologic studies of Gulf War veterans are available elsewhere (CDC, 1999; IOM, 1999a).

The Iowa Study

The "Iowa study," a major population-based study of U.S. Gulf War veterans, was a cross-sectional survey of a representative sample of 4,886 military personnel 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 from all branches of service who either were still serving or had left service. The sample was randomly selected from, and therefore representative of, about 29,000 military personnel. Of the eligible study subjects, 3,695 (76 percent) completed a telephone interview. Study subjects were divided into four groups, two that had been deployed to the Gulf War and two that had not been deployed to the Gulf War. Trained examiners using standardized questions, instruments, and scales interviewed the subjects.[9] The two groups of Gulf War military personnel reported roughly twice the prevalence of symptoms suggestive of the following conditions: fibromyalgia, cognitive dysfunction, depression, alcohol abuse, asthma, posttrau-

[9]Sources of questions included the National Health Interview Survey, the Behavioral Risk Factor Surveillance Survey, the National Medical Expenditures Survey, the Primary Care Evaluation of Mental Disorders, the Brief Symptom Inventory, the CAGE questionnaire, the PTSD (Posttraumatic Stress Disorder) Checklist—Military, the Centers for Disease Control and Prevention Chronic Fatigue Syndrome Questionnaire, the Chalder Fatigue Scale, the American Thoracic Society questionnaire, the Sickness Impact Profile, and questions to assess fibromyalgia, sexual functioning, and military exposures.

TABLE 2.3 Major Studies of Gulf War Veterans' Symptoms and Syndromes

Reference	Subjects/ Controls (*n*)	Study Design	Military Branch and Status	Response Rate (%)	Major Findings
Population-Based Studies					
Iowa Persian Gulf Study Group, 1997	1,896/1,799	Population-based survey	All U.S. branches and duty status	76	Symptoms (subjects vs. controls) Fibromyalgia: 19.2% vs. 9.6% Cognitive dysfunction: 18.7% vs. 7.6% Depression: 17.0% vs. 10.9%
Goss Gilroy, 1998	3,113/3,439	Survey	All Canadian Gulf War veterans	64.5	Symptoms Chronic fatigue (OR = 5.27) Cognitive dysfunction (OR = 4.36) Multiple chemical sensitivity (OR = 4.01)
Unwin et al., 1999; Ismail et al., 1999	2,961/2,620, 2,614[a]	Population-based survey, factor analysis	U.K. Gulf War veterans	65.1	Symptoms Fatigue (OR = 2.2) Posttraumatic stress (OR = 2.6) Psychological distress (OR = 1.6) Three factors (mood, respiratory system, peripheral nervous system) not unique to Gulf War veterans

Other Epidemiologic Studies					
Haley et al., 1997b	249/no controls	Survey, factor analysis	Navy reserve	41	25% have one of six syndromes: impaired cognition, confusion–ataxia, arthro-myo-neuropathy, phobia–apraxia, fever–adenopathy, weakness–incontinence
Fukuda et al., 1998	1,163/2,538	Survey, clinical exam, factor analysis	Air Force National Guard and 3 other Air Force units	35–70	31 of 33 symptoms significantly more prevalent in Gulf War veterans; defined case as 1 or more symptoms from 2 of 3 categories: fatigue, mood-cognition, musculoskeletal; case not unique to Gulf War veterans
Proctor et al., 1998	300[b]/48	Survey or clinical interview	All U.S. branches and duty status	38–62	PTSD diagnosis: 5–7% vs. 0% Dermatological symptoms (OR = 9.6, 6.9)[b] Gastrointestinal symptoms (OR = 8.0, 5.8)[b] Neuropsychological symptoms (OR = 6.4, 5.2)[b]

NOTE: OR = odds ratio; PTSD = posttraumatic stress disorder.

[a]Two comparison groups (Bosnia, Gulf era).
[b]The 300 Gulf War veterans came from two study groups—one from Ft. Devens and the other from New Orleans. The control group was deployed to Germany.

matic stress disorder (PTSD), sexual discomfort, or chronic fatigue (Table 2.4).[10] Furthermore, on a standardized instrument for assessing functioning (the Medical Outcome Study's 36-item questionnaire known as the Short Form-36, or SF-36), Gulf War veterans displayed significantly lower scores across all eight subscales for physical and mental health. These subscales profile different aspects of quality of life. The subscales for bodily pain, general health, and vitality showed the greatest absolute differences between deployed and nondeployed veterans. In short, this large, well-controlled study demonstrated that certain sets of symptoms are more frequent and quality of life is poorer among Gulf War veterans than among nondeployed military controls.

Symptom clustering. The Iowa study was the first major population-based study to group together sets of symptoms into categories suggestive of existing syndromes or disorders, such as fibromyalgia or depression. The Iowa study did not search for new syndromes. However, its finding of such higher prevalence of symptom groups suggestive of fibromyalgia, depression, and cognitive dysfunction (see Table 2.4) motivated subsequent researchers to examine the potential for a new syndrome that would group together and classify veterans' symptoms.

Exposure–symptom relationships. The Iowa study assessed exposure–symptom relationships by asking veterans to report on their past exposures. Researchers found that many of the self-reported exposures were significantly associated with many different health conditions. For example, symptoms of cognitive dysfunction were found to have been associated with self-reports of exposure to solvents or petrochemicals, smoke or combustion products, sources of lead from fuels, pesticides, ionizing or nonionizing radiation, chemical warfare agents, use of pyridostigmine, infectious agents, and physical trauma. A similar set of exposures also was associated with symptoms of depression or fibromyalgia. The study concluded that no single exposure to any specific agent was related to the conditions that the authors found to be more prevalent in Gulf War veterans.

The Canadian Study

The findings of a 1997 survey mailed to the entire cohort of Canadian Gulf War veterans were similar to those from the Iowa study. In this study, Canadian forces deployed to the Gulf War (n = 3,113) were compared with Canadian forces deployed elsewhere (n = 3,439) during the same period (Goss Gilroy, 1998). Of the Gulf War veterans responding, 2,924 were male and 189 were female. Deployed forces had significantly higher rates than controls of self-

[10]The conditions listed were not diagnosed because no clinical examinations were performed. Rather, before conducting their telephone survey, researchers grouped together sets of symptoms from their symptom checklists into a priori categories of diseases or disorders. After a veteran identified him- or herself as having the requisite set of symptoms, researchers analyzing responses considered the veteran as having symptoms "suggestive" of or consistent with a particular disorder, but not as having a formal diagnosis of the disorder.

reported chronic conditions and symptoms of a variety of derived clinical outcomes[11] (chronic fatigue, cognitive dysfunction, multiple chemical sensitivities, major depression, PTSD, chronic dysphoria, anxiety, fibromyalgia, and respiratory diseases). The greatest differences between deployed and nondeployed forces were in the first three outcomes. The symptom grouping with the highest overall prevalence was cognitive dysfunction, which occurred in 34–40 percent of Gulf War veterans compared with 10–15 percent of control veterans. Gulf War veterans also reported significantly more visits to health care practitioners, greater dissatisfaction with health, and greater reductions in recent activity because of health than control veterans.

Symptom clustering. The Canadian study did not search for potentially new syndromes.

Exposure–symptom relationships. In Canadian Gulf War veterans, the greatest number of symptom groupings were associated with self-reported exposures to psychological stressors and physical trauma. Several symptom groupings also were associated with exposure to chemical warfare agents, nonroutine immunizations, sources of infectious diseases, and ionizing or nonionizing radiation. Nevertheless, a subset of Canadian veterans who could not have been exposed to many of the agents, because they were based at sea, reported symptoms as frequently as did land-based veterans in this study.

TABLE 2.4 Results of the Iowa Study

Symptoms (in order of frequency)[a]	Prevalence in Gulf War Veterans (%)	Prevalence in Non-Gulf War Veterans (%)
Fibromyalgia	19.2	9.6
Cognitive dysfunction	18.7	7.6
Alcohol abuse	17.4	12.6
Depression	17.0	10.9
Asthma	7.2	4.1
PTSD	1.9	0.8
Sexual discomfort	1.5	1.1
Chronic fatigue	1.3	0.3

[a]Based on a survey instrument designed by investigators to incorporate structured instruments and standardized questions.

SOURCE: Iowa Persian Gulf Study Group, 1997.

[11]Several of the reported health conditions or symptoms were combined to form clinically meaningful outcomes (Goss Gilroy, 1998).

The U.K. Study

Unwin and collaborators (1999) investigated the health of servicemen from the United Kingdom in a population-based study. This study is especially useful because the researchers conducted a random sample of the entire U.K. contingent of about 53,000 personnel deployed to the Persian Gulf[12] and used two comparison groups. One of the comparison groups was deployed to the conflict in Bosnia (n = 4,250), making this study 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 United Kingdom over the same time frame (n = 4,246). Through a mailed questionnaire, the investigators asked about symptoms (50 items), medical disorders (39 items), and functional capacity, among other topics. The findings for the Gulf War cohort and comparison cohorts were compared through calculation of odds ratios. The study controlled for potential confounding factors (including sociodemographic and life-style factors) by logistic regression analysis. Only male veterans' results were analyzed, however, because female veterans' roles and symptoms were distinct enough to warrant separate consideration.

The U.K. Gulf War-deployed veterans (n = 4,248) reported higher prevalence of symptoms and diminished functioning than did both comparison groups. Gulf War veterans were two to three times more likely than comparison subjects to have met symptom-based criteria for chronic fatigue, posttraumatic stress reaction, and "chronic multisymptom illness" (the label for the first case definition[13] developed by Centers for Disease Control and Prevention [CDC] researchers to probe for the existence of a potentially new syndrome among Gulf War veterans) (Fukuda et al., 1998). That the Bosnia cohort in the U.K. study, which was deployed to a combat setting, reported fewer symptoms than the Gulf War cohort, suggests that combat deployment per se does not account for higher symptom reporting.

Symptom clustering. In a companion study using the U.K. data set, Ismail and colleagues (1999) set out to determine whether the symptoms that occurred with heightened prevalence in U.K. Gulf War veterans constitute a new syndrome. By applying factor analysis, the researchers were able to identify three fundamental factors, which they classified as mood, respiratory system, and peripheral nervous system, according to the types of symptoms that contributed to each factor. The pattern of symptom reporting by Gulf War veterans differed little from the patterns of Bosnia and Gulf War era comparison groups,

[12]U.K. military personnel in the Gulf War were somewhat different from U.S. personnel in terms of demographics, combat experience, and exposures to certain agents (U.K. Ministry of Defense, 2000).

[13]A case is defined as having one or more chronic symptoms from at least two of these three categories: fatigue, mood–cognition (e.g., feeling depressed, difficulty remembering or concentrating), and musculoskeletal (joint pain, joint stiffness, or muscle pain). This case definition was developed as a research tool in order to organize veterans' unexplained symptoms into a potentially new syndrome, as explained elsewhere in this section.

although the Gulf War cohort had a higher frequency of symptom reporting. Further, this study did not identify in this cohort of Gulf War veterans the six factors characterized by Haley and colleagues (1997b) in a separate factor analysis study described in the next section. The authors interpreted their results as evidence against the existence of a unique Gulf War syndrome.

Exposure–symptom relationships. In the U.K. Gulf War cohort, most self-reported exposures were associated with all of the health outcomes, which was also true for the two comparison cohorts (Unwin et al., 1999). The authors interpreted these findings as evidence that the exposures were not uniquely associated with Gulf War-related illnesses. Within the Gulf War cohort, two vaccine-related exposures—vaccination against biological warfare agents and receiving multiple vaccinations—were associated with meeting the case definition for the chronic multisymptom illness developed by CDC researchers. A recent analysis of the data on a subcohort of U.K. veterans found that receiving multiple vaccinations during deployment was associated with five of the six health outcomes examined (including multisymptom illness as defined by the CDC) (Hotopf et al., 2000). Vaccine-related findings are discussed in greater detail in Chapter 7.

Other Studies of Veterans' Symptoms and General Health Status

One of the first epidemiologic studies of U.S. Gulf War veterans was of more than 4,000 active duty and reserve personnel from the states of Pennsylvania and Hawaii (Stretch et al., 1995). Veterans deployed to the Gulf reported higher prevalence than nondeployed veterans of 21 out of 23 symptoms on a symptom checklist (although the total response rate was only 31 percent). Overall, deployed veterans were about two to four times more likely than nondeployed veterans to report each symptom.

The symptom experience of two cohorts of Gulf War veterans from Massachusetts (Ft. Devens) and New Orleans was studied by Proctor and colleagues (1998). In comparison with veterans deployed to Germany during the Gulf War era, stratified random samples of both Gulf War cohorts reported elevated prevalence of 51 out of 52 items on a health symptom checklist. The greatest differences in prevalence of reported symptoms were for dermatological (e.g., skin rash, eczema, skin allergies), neuropsychological (e.g., difficulty concentrating, difficulty learning new material), and gastrointestinal symptoms (e.g., stomach cramps, excessive gas). The study's nearly 300 subjects represented a stratified random sample of 2,949 troops from Ft. Devens and 928 troops from New Orleans, both consisting of active duty, reserve, and National Guard troops. These cohorts were also the focus of several in-depth studies of stress-related disorders (see discussion later in this chapter).

Women veterans from the Air Force were studied by Pierce (1997). The study examined a stratified sample of 525 women (active duty, guard, and reserve) drawn from all 88,415 women who served in the Air Force during the Gulf War era. Women deployed to the Gulf War, in comparison with women

deployed elsewhere, more frequently reported the following symptoms: skin rash, cough, depression, unintentional weight loss, insomnia, and memory problems. The pattern of symptom reporting is similar to that reported by men and women who participated in the Iowa study. In addition, women deployed to the Gulf War were more likely than controls to report gender-specific problems, such as breast cysts and lumps, and abnormal cervical cytology (Pierce, 1997).

The first published study to search for new syndromes was conducted by Haley and collaborators (1997b). They studied a battalion of naval reservists called to active duty for the Gulf War (n = 249). More than half of the battalion had left the military by the time of the study. Of those participating, 70 percent reported having had a serious health problem since returning from the Gulf War, while about 30 percent reported having no serious health problems. The study was the first to cluster symptoms into new syndromes by applying factor analysis (see above). Through standardized symptom questionnaires and two-stage factor analysis, the investigators defined what they considered to be either six separate syndromes or six variants of a single syndrome, which they labeled impaired cognition, confusion–ataxia, arthromyoneuropathy, phobia–apraxia, fever–adenopathy, and weakness–incontinence. One-quarter of the veterans in this uncontrolled study (n = 63) were classified as having one of the six syndromes. The first three of the syndromes had the strongest factor clustering of symptoms (see earlier discussion of factor analysis and Chapter 6).

In a follow-up study of the same cohort, Haley and colleagues (1997a) used a case-control design to examine neurological function. They chose as cases the 23 veterans who had scored highest on the three syndromes with the strongest factor clustering. The results of extensive neurological and neuropsychological testing, demonstrated that cases had significantly greater evidence of neurological dysfunction when compared with two small groups of healthy controls from the same battalion.[14] Investigators concluded that the three syndromes, derived from factor analysis of symptoms, may signify variant forms of expression of a generalized injury to the nervous system.[15] In a subsequent study, cases with one of the three syndromes were more likely than healthy controls to exhibit vestibular dysfunction (Roland et al., 2000).

The three syndromes identified by Haley and colleagues (1997b) were the focus of a companion case-control study that examined their relationship to self-reported exposures to neurotoxins. The study tested the hypothesis that exposure

[14]One group of healthy controls (n = 10) was deployed to Gulf War, whereas the other group (n = 10) was not.

[15]Neuropsychological or neurological impairments have been the focus of several smaller studies as well. Some found subtle changes in nerve conduction velocity and cold sensation (Jamal et al., 1996) and in certain tests of finger dexterity and executive functioning (Axelrod and Milner, 1997), while other studies found no significant differences in measures of nerve conduction and neuromuscular functioning (Amato et al., 1997) or neuropsychological performance (Goldstein et al., 1996). Numerous ongoing studies are designed to probe further whether Gulf War veterans have measurable impairments of neurological or neuropsychological performance (CDC, 1999; Research Working Group, 1999).

to organophosphates and related chemicals that inhibit cholinesterase is responsible for the three nervous system-based syndromes (Haley and Kurt, 1997). Each of the syndromes was associated with a distinct set of risk factors. The impaired cognition syndrome was found, through multiple logistic regression, to be associated with having a job in security and wearing flea-and-tick collars. The second syndrome, confusion–ataxia, was associated with self-reports of having been involved in a chemical weapons attack and with self-reports of having advanced adverse effects from pyridostigmine bromide (PB).[16] Finally, the third syndrome, arthromyoneuropathy, was associated with higher scores on the scale of advanced adverse effects from PB, as well as with an index created by the investigators to enable veterans to self-report the amount and frequency of their use of government-issued insect repellent. The authors concluded that some Gulf War veterans had delayed, chronic nervous system syndromes as a result of exposure to combinations of neurotoxic chemicals (Haley and Kurt, 1997).

Another study by Haley and collaborators (1999) examined whether genetic susceptibility could play a role in placing certain veterans at risk for neurological damage from organophosphate chemicals. They hypothesized that neurological symptoms in ill veterans might be explained by their having genetic polymorphisms (genetic variations) in metabolizing enzymes. The polymorphism would impair their ability to rapidly detoxify organophosphates (e.g., sarin, soman, and certain pesticides). This study is described and assessed in Chapter 6. The investigators studied 45 veterans, 25 of whom had chronic neurological symptoms as identified through their earlier factor analysis study and 20 of whom were healthy controls from the same battalion. They measured blood levels of butyrylcholinesterase (BuChE) and two types, or allozymes, of paraoxonase/arylesterase 1 (PON1). The genotypes encoding the allozymes were also studied. Investigators found that veterans who were ill had levels of blood BuChE similar to control subjects; however, ill veterans had lower levels of type Q PON1, the allozyme that hydrolyzes sarin rapidly. They also were more likely to have the type R genotype, which encodes the allozyme with low hydrolyzing activity for sarin. The authors interpreted their findings as supporting their earlier studies that neurological symptoms in susceptible Gulf War veterans were caused by exposure to environmental chemicals (see discussion in Chapters 5 and 6). This work requires further investigation and independent confirmation.

A large study by Fukuda and colleagues (1998) used factor analysis and other methods to assess the health status of Gulf War veterans. By studying an Air Force National Guard unit from Pennsylvania and three comparison Air Force populations, the investigators aimed to organize symptoms into a case definition and to carry out clinical evaluations on a subset of veterans. Of 3,701 veterans surveyed, those deployed to the Gulf War experienced higher prevalence of chronic symptoms (33 of 35 symptoms with more than 6-month dura-

[16]The scale for adverse effects of PB was developed by the investigators to measure less common adverse effects (e.g., excessive sweating, tearing, chest tightness, nausea, muscle twitching, muscle cramps, headache, pounding heartbeat).

tion were reported to be more prevalent) in comparison with nondeployed veterans. The authors then used two alternative methods to derive a case definition: factor analysis and a clinical approach. Since both approaches yielded similar case definitions, the investigators chose the latter for its simplicity of application in research.

The authors defined a case of chronic multisymptom illness as having one or more chronic symptoms from at least two of these three categories: fatigue, mood–cognition (e.g., feeling depressed, difficulty remembering or concentrating), and musculoskeletal (joint pain, joint stiffness, or muscle pain). According to this definition, 39 percent of Gulf War-deployed veterans versus 14 percent of nondeployed veterans had a mild-to-moderate case, whereas 6 percent versus 0.7 percent, respectively, had a severe case. Based on a total of 158 clinical examinations performed on one unit, there were no abnormal physical or laboratory findings among those who met the case definition. Cases reported significantly lower functioning and well-being.

A sizable fraction (14 percent) of nondeployed veterans also met the mild-to-moderate case definition. The investigators therefore concluded that their case definition could not uniquely characterize Gulf War veterans with unexplained illnesses (Fukuda et al., 1998). The study, however, had several limitations, the most important of which was its coverage of only active Air Force personnel (several years after the Gulf War), which limits its generalizability to other branches of service, as well as to those who left the service possibly due to illness.

To assess risk factors, the authors performed clinical evaluations on a subset of veterans (n = 158), all of whom volunteered for the evaluation and came from the index unit of the Pennsylvania Air Force National Guard. Forty-five percent of this unit had been deployed to the Gulf War. Overall, there was a dearth of abnormal findings from blood, stool, and urine testing among those who met the case definition for chronic multisymptom illness. There were no differences between cases and noncases in the proportion that seroreacted to botulinum toxin, anthrax protective antigen, and leishmanial antigens, among other antigens. This was the only study to have assessed exposures (mostly to infectious diseases) via laboratory testing, as opposed to self-reports, but the sample undergoing clinical evaluation was relatively small and restricted to Air Force National Guard members.

EPIDEMIOLOGIC STUDIES OF SPECIFIC HEALTH ENDPOINTS

Mortality Studies

A large mortality study of nearly all Gulf War-deployed veterans identified no excess postwar mortality, with the exception of motor vehicle accidents (Kang and Bullman, 1996). The study examined mortality patterns through 1993 using two databases, the VA's Beneficiary Identification and Records Locator

Subsystem and deaths reported to the Social Security Administration.[17] It compared deployed veterans with a similarly sized cohort of veterans who did not serve in the Gulf War. A further analysis extended the mortality data through 1997 with no change in the results (Kang and Bullman, 1999).

A second mortality study of active duty military personnel focused exclusively on the Gulf War period. This study compared noncombat mortality rates among troops stationed in the Gulf War versus troops on active duty elsewhere. There was no excess noncombat mortality in deployed veterans, except for unintentional injury (due to vehicle accidents and other causes; Writer et al., 1996).

The principal limitation of published mortality studies is the short duration of follow-up observation. More time must elapse before excess mortality would be expected from illnesses with long latency (e.g., cancer) or a gradually deteriorating course (e.g., multiple sclerosis).[18] An ongoing, long-term study of all U.K. veterans of the Gulf War in relation to contemporaneous controls is assessing the incidence of cancer and all-cause mortality (Cherry and Macfarlane, 1999).

Hospitalization Studies

The risk of hospitalization was the subject of two large studies of active duty personnel discharged from DoD hospitals before and after the Gulf War. The first study, compared almost 550,000 Gulf War veterans with almost 620,000 nondeployed veterans and found no significant and consistent differences in hospitalizations after the war (Gray et al., 1996). Before the Gulf War, from 1988 to 1990, those subsequently deployed to the Gulf were at lower risk of hospitalization than their nondeployed counterparts, probably due to the healthy-warrior effect. In order to permit valid "before-versus-after" comparisons, the investigators used statistical methods to remove bias introduced by the healthy-warrior effect (also "healthy-worker effect"; see Chapter 3).

A second hospitalization study reexamined the same data set of active duty personnel discharged from DoD hospitals to search for excess hospital admissions because of unexplained illnesses. The authors reasoned that the first study

[17]The degree of completeness using these record systems was assessed by a validation study using state vital statistics data. Ascertainment was estimated at 89 percent of all deaths in the Gulf War cohort and comparison group.

[18]Critics assert that the mortality study by Kang and Bullman (1996) made errors in calculating confidence intervals around mortality rates and did not adequately account for the "healthy-warrior effect" (i.e., the possibility that troops mobilized to the Gulf War were healthier than nondeployed troops, thereby biasing the study toward not finding a mortality difference) (Haley, 1998). The study authors disagreed with this assertion and demonstrated that other statistical techniques, recommended by Haley, had negligible impact on their confidence intervals (Kang and Bullman, 1998). To counter the charge of selection bias by Haley (1998), the study authors point out that effects of any potential selection bias are minimal because they found no differences in mortality risk between troops mobilized to sites other than the Persian Gulf and troops not mobilized at all (Kang and Bullman, 1998).

might have missed hospitalizations for a new or poorly recognized syndrome(s). Hospital discharge coding might have inconsistently classified such hospitalizations under many different diagnoses so as to mask an effect, if one were present. The second study operationally defined unexplained illnesses as diagnoses falling under several catch-all *International Classification of Diseases, Ninth Revision—Clinical Modification* (ICD-9-CM) diagnostic categories entailing nonspecific infections and other ill-defined conditions. After adjusting for hospitalizations only for evaluation (as opposed to treatment) under the DoD registry program, the authors found no significant differences between deployed and nondeployed active duty military (Knoke and Gray, 1998).

These hospitalization studies provide some reassurance that excess hospitalizations did not occur among veterans of the Gulf War remaining on active duty through 1993. Like the mortality studies, however, these studies do not capture illnesses that might have longer latency (e.g., cancer) or illnesses in individuals separated from the military and admitted to nonmilitary hospitals (VA and civilian hospitals) (Haley, 1998). The studies did not measure the utilization of outpatient treatment and would not have detected illnesses unless that did not require hospitalization (Gray et al., 1996; Knoke and Gray, 1998).

Studies of Birth Defects and Reproductive Outcomes

Several studies have not identified an excess of birth defects in offspring of deployed versus nondeployed veterans. A small study of two Mississippi National Guard units (n = 282) deployed to the Persian Gulf found no excess rate of birth defects in their children compared with expected rates from surveillance systems and previous surveys (Penman et al., 1996). A much larger study of all live births in military hospitals (n = 75,000) from 1991 to 1993 included a comparison population of nondeployed personnel. The risk of birth defects in children of Gulf War personnel was the same as in the control population (Cowan et al., 1997). This important study, the largest to date on birth defects, was limited to military hospitals, thereby excluding those ineligible for care in military hospitals (i.e., members of the National Guard, reserves, and those who left the military over the course of study). National Guard and reserve troops, as noted earlier in this chapter, constituted a relatively high percentage of U.S. troops deployed to the Persian Gulf (Table 2.1). Anecdotal reports of an excess of Goldenhar's syndrome, a rare congenital anomaly affecting the development of facial structures, prompted another study of birth defects. Since this birth defect is not specifically coded for in reporting birth defects, the study reviewed medical records of all listings in several more inclusive birth defect categories under which this syndrome would have been subsumed. Araneta and colleagues (1997) found too few cases of Goldenhar's syndrome from which to draw definitive conclusions.

Several ongoing studies are addressing the limitations of previous studies. Population-based studies to capture births in all hospitals—both military and civilian—are under way in the United States and the United Kingdom. A large U.S. study will pool birth defect data across several states using statewide birth

certificates matched with military records (Araneta et al., 1999). Another ongoing study in the United Kingdom probes the prevalence of birth defects, problems in reproduction and fertility, exposure history and cancer in children. This study covers all Gulf War veterans and Gulf War era controls, a total of 106,000 veterans (Doyle et al., 1999).

Studies of Stress-Related Disorders

Two population-based epidemiologic studies described earlier (Iowa Persian Gulf Study Group, 1997; Goss Gilroy, 1998) detected a significant elevation in the self-reported prevalence of symptoms that may indicate posttraumatic stress disorder (an anxiety disorder) and depression.[19] In the Iowa study, 17 percent of Gulf War veterans reported symptoms of depression and 1.9 percent reported symptoms of PTSD. These figures were significantly higher than those for controls, whose prevalences were 11 and 0.8 percent, respectively (Table 2.4). The third population-based study found that Gulf War veterans from the United Kingdom were about 2½ times more likely than controls to have symptoms of PTSD. In this study, there were no significant differences in the levels of depression between deployed veterans and controls (Unwin et al., 1999).

In a study of military personnel (n = 16,167) from Pennsylvania and Hawaii (described earlier), 8–9 percent of deployed veterans met criteria for PTSD symptoms, based on self-reported symptom checklists, in comparison with 1–2 percent of nondeployed veterans (Stretch et al., 1996). Similarly, a small study found higher PTSD scores in deployed versus nondeployed veterans (Perconte et al., 1993a).

Sutker and colleagues (1993) compared 215 National Guard and Army reserve veterans who were deployed to the Persian Gulf with 60 veterans from the same unit who were activated but not deployed overseas. None had sought mental health treatment. The investigators found 16 to 24 percent of war zone–exposed troops had symptoms of distress that suggested depression and/or PTSD. Those who reported higher levels of stress had greater severity of PTSD and more health complaints than veterans who had low self-reported stress or no war-zone stress. Similarly, PTSD symptoms or diagnoses were more likely in groups of Gulf War veterans with combat exposure or injury (Baker et al., 1997; Labbate et al., 1998; Wolfe et al., 1998), those of female gender (Wolfe et al., 1993), veterans who had been exposed to missile attack (Perconte et al., 1993b), and those that had grave registration duties (Sutker et al., 1994).

[19]Most epidemiologic studies of veterans have assessed the prevalence of self-reported symptoms of PTSD by asking subjects to fill out one or more validated psychometric scales, such as the Mississippi Scale for Combat-Related PTSD or the PTSD Checklist—Military. Psychometric scales of PTSD, while useful as screening tools for approximating a PTSD diagnosis, are not deemed to be diagnostic by themselves (Keane et al., 1988; Kulka et al., 1991).

A study by Engel and colleagues (1999) is one of only a few that used a clinician-administered diagnostic instrument rather than self-reported symptom scales to assess the presence of psychiatric disorders. Researchers compiled diagnoses from among all Gulf War veterans (n = 13,161) who sought health examinations through the DoD registry during its first year of operation (1994–1995). Study authors used the Structured Clinical Interview for the *Diagnostic and Statistical Manual of Mental Disorders*, Third Edition (DSM-III-R [SCID-NP]) to explore a range of possible psychiatric disorders and the Clinician-Administered PTSD Scale to explore PTSD. Both of these measures have been psychometrically validated on combat veterans, making this study methodologically stronger than many of the previous investigations. Unfortunately, the study did not employ a control or comparison group and, in using a treatment-seeking population, was not, by design, representative of the Gulf War veteran population. The authors found that 37 percent of the veterans met criteria for at least one psychiatric disorder. About 13 percent of the entire sample met diagnostic criteria for mood disorders, 14 percent met criteria for somatoform disorders,[20] and 6 percent met criteria for current PTSD. A study on a subset of this cohort (n = 131) referred for specialty evaluation found significant associations with PTSD and somatoform disorder among those reporting traumatic events (such as handling dead bodies) (Labbate et al., 1998). The authors of this smaller study concluded that at least some veterans with unexplained physical symptoms might be suffering the consequences of combat trauma.

The most methodologically rigorous study to have undertaken structured clinical interviews (in addition to PTSD questionnaires) found a current diagnosis of PTSD in 5–7 percent of two groups of deployed veterans (n = 206), compared with none in a control group deployed to Germany (n = 48) (Wolfe et al., 1999).[21] Investigators used a stratified random sampling strategy to identify participants from two cohorts of Gulf War veterans from New England and New Orleans. The study also found similarly elevated rates of current major depressive disorder and dysthymia (two distinct types of depression) but did not find elevated rates of somatoform disorders. Yet nearly two-thirds of veterans reporting health symptoms in the moderate to high range had no current diagnosis of a mental disorder such as PTSD or major depressive disorder.[22] The authors concluded that, although psychiatric illness is associated with some Gulf War

[20]This term encompasses a variety of disorders in which the patients have multiple physical symptoms that are not explained by a known medical disease or condition, by the effects of a substance, or by another mental disorder. The symptoms cause clinically significant distress or impaired functioning (APA, 1994).

[21]Four percent of one of the deployed groups (the Ft. Devens cohort) was found to have PTSD symptoms, as measured by psychometric scale within 5 days of returning from the Gulf War, suggesting that PTSD symptoms are chronic (Wolfe et al., 1998), a finding also supported in an uncontrolled study that followed a small cohort for 2 years (Southwick et al., 1995).

[22]About 40 percent also had no lifetime history of these disorders (Wolfe et al., 1999).

health complaints, such illnesses do not entirely account for the full range and extent of Gulf War veterans' symptom reporting.

Studies of Infectious Disease, Gastrointestinal Symptoms, and Testicular Cancer

During the Gulf War, the occurrence of infectious diseases was lower than expected (Hyams et al., 1995). The most common infectious disease among U.S. troops was diarrheal disease caused by the bacterial pathogens *Escherichia coli* and *Shigella sonnei*, as detected by stool cultures (Hyams et al., 1991). Almost 60 percent of troops responding to a questionnaire reported at least one episode of diarrheal disease within an average of 2 months in Saudi Arabia (Hyams et al., 1991). Upper respiratory infections also were frequent (Hyams et al., 1995). Finally, 19 cases of cutaneous leishmaniasis and 12 cases of a variant of visceral leishmaniasis have been reported among U.S. Gulf War veterans.[23] The latter is an unusual finding because the etiological agent found in veterans' tissue samples—the protozoan parasite *Leishmania tropica,* transmitted by sandflies—is not endemic to the Persian Gulf area and is usually associated with cutaneous leishmaniasis (CDC, 1992; Magill et al., 1993; Hyams et al., 1995). Because veterans' symptoms (e.g., fever, lymphadenopathy, and hepatosplenomegaly) were milder than symptoms of classic visceral leishmaniasis, the condition was given the name viscerotropic leishmaniasis. Even though visceral leishmaniasis and its variants are chronic infectious diseases, the cases were considered too few, and classic signs and symptoms too readily detectable at physical examination, to account for the much more frequent occurrence of unexplained illnesses in veterans (Hyams et al., 1995; PAC, 1996). Further, in the controlled study of Gulf War veterans by Fukuda and colleagues (1998), none of the eight participants who seroreacted to leishmanial antigens met the study's case definition for a severe case of unexplained illness, which suggests that viscerotropic leishmaniasis is distinct from veterans' unexplained illnesses. However, some individuals with visceral or viscerotropic leishmaniasis can present with nonspecific symptoms (fatigue, low-grade fever, gastrointestinal symptoms) that are consistent with those seen in veterans with unexplained illnesses. Further research is required (NIH, 1994).

Gastrointestinal complaints, as noted earlier, are somewhat common among veterans in the DoD and VA registries (Joseph, 1997; Murphy et al., 1999). In the study reported earlier by Proctor and colleagues (1998), gastrointestinal symptoms were among the symptoms with greatest prevalence differences between deployed and nondeployed veterans. One study investigated a host of gastrointestinal symptoms in a National Guard unit (n = 136). Excessive gas,

[23]Leishmaniasis is any variety of diseases affecting the skin (cutaneous leishmaniasis), mucous membranes, and internal organs (visceral leishmaniasis, caused by infection with single-celled parasites called leishmania. It is transmitted from infected animals or people to new hosts by the bites of sand flies (Clayman, 1989).

loose stool, incomplete rectal evacuation, and abdominal pain were more prevalent during and after the war in deployed than in nondeployed veterans from the same unit (Sostek et al., 1996). The results were based on a 64-item questionnaire administered after the war. Subjects reported that their gastrointestinal complaints began in the Gulf and persisted after return to the United States.

Over the last 5 months of 1991, hospitalizations for testicular cancer were slightly elevated in a large study of active duty deployed versus nondeployed veterans (Gray et al., 1996). In a follow-up study, the investigators extended their analysis through 1996. They replicated their earlier finding, but also found that by 4 years after the war, the cumulative risk of testicular cancer was similar for the two groups of veterans (Knoke et al., 1998). They attributed the transient increase in testicular cancer immediately after the war to regression to the mean because of the healthy-servicemen selection effect and to deferring care during deployment (during which time they would not have had the opportunity for diagnosis and treatment).

LIMITATIONS OF PAST STUDIES AND ONGOING STUDIES

The epidemiologic studies of Gulf War veterans summarized above have contributed greatly to our understanding of veterans' symptoms, but they are beset by limitations commonly encountered with epidemiologic studies. A major limitation is representativeness; most studies focus on groups that are not representative of all Gulf War veterans, by virtue of either their military duties and location during deployment; their military status during the war (active duty, reserves or National Guard); their military status after the war (active duty, reserves, discharged); their branch of service (Army, Navy, Air Force, Marines); or ease of ascertainment (IOM, 1999a). The Iowa study, with its population-based design, had the broadest coverage of U.S. Gulf War veterans. Although it is considered the most representative, the cohort contained few members of racial and ethnic minorities (Iowa Persian Gulf Study Group, 1997). The findings from population-based studies from Canada (Goss Gilroy, 1998) and the United Kingdom (Unwin et al., 1999) are generally consistent with U.S. studies.

Other limitations of epidemiologic studies include small sample size, low participation rates that could result in selection bias in some studies, and recall bias.[24] The potential for recall bias is particularly important because most studies rely on self-reporting of symptoms and exposures years after the event, rather than on biological measures (Joellenbeck et al., 1998). Additionally, studies may be too narrow in their assessment of health status. The measurement instruments may have been too insensitive to have detected abnormalities affecting deployed

[24]Selection bias would occur if Gulf War veterans who are symptomatic choose to participate in a study more frequently than those who are not symptomatic. Recall bias would occur if Gulf War veterans who are symptomatic tend to overestimate their previous exposures in comparison with veterans who are not symptomatic (see Chapter 3).

veterans. Finally, the period of investigation has, of necessity, been too brief to detect health outcomes that have a long latency period or require many years to progress to the point where disability, hospitalization, or death occurs. Virtually all U.S. studies are cross sectional, which limits the opportunity to learn about symptom duration and chronicity, latency of onset (especially for health conditions with a long-term latency such as cancer), and prognosis. In light of the limitations surrounding studies of veterans' health, a recent Institute of Medicine (IOM) panel recommended a prospective cohort study of Gulf War veterans (IOM, 1999a).

A major study currently in progress by the VA may overcome some of the limitations of past studies. The study, mandated by Public Law 103-446, is a retrospective cohort study. Its purpose is to estimate the prevalence of symptoms and other health outcomes in Gulf War veterans versus non–Gulf War veterans.[25] This population-based survey has three phases. The first phase is a questionnaire mailed to a total of 30,000 veterans. The second phase will validate self-reported data with medical record review and analyze characteristics of those who do not respond to the mailed survey. The third phase is a comprehensive medical examination and laboratory testing of a random sample of 2,000 veterans drawn from both the Gulf War and the comparison group (Research Working Group, 1998). The purpose of the third phase is to establish diagnoses that will make it possible to see what proportion of self-reported symptoms are due to established diseases rather than unexplained illnesses.[26]

A major problem for most epidemiologic studies of Gulf War veterans is the lack of biological measures of exposure to potentially harmful agents. Reliance on self-reported exposures, often taking place years earlier, lacks external verification and is subject to recall bias, a potential problem that affects many retrospective epidemiologic studies. Further, self-reports of exposure may be complicated by recall of perceived—rather than actual—exposures (e.g., because of the sensitivity of the monitors, many false alarms may have been perceived as chemical warfare agent exposure). Enhanced record keeping and monitoring of the environment during and after the Gulf War would have averted this problem. Indeed, many expert panels have recommended efforts to improve record keeping and environmental monitoring in future deployments (e.g., IOM, 1999b; NRC, 2000a,b,c).

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[25]Health outcomes include reproductive outcomes in spouses and birth defects in children.

[26]After the committee completed its deliberations and submitted its report for peer review, the first two phases of the VA study were published (Kang et al., 2000). This study found that Gulf War veterans, in comparison with non-Gulf War veterans, reported higher prevalence of functional impairment, health care utilization, symptoms, and medical conditions. The nature of health concerns and their prevalence were similar to those of U.K. veterans (Unwin et al., 1999). The VA study surveyed veterans about their self-reported exposures in the Gulf War, but did not perform any analyses to determine whether self-reported exposures were related to symptoms and health reporting. The third phase of the VA study has yet to be published.

CONCLUSIONS

This chapter provides an overview of the rapidly growing body of published studies on the health of Gulf War veterans. Many of the studies described in this chapter have been released in the past few years, and the largest U.S. study of veterans' health has yet to be completed. However, current research demonstrates that Gulf War veterans report more symptoms than their nondeployed counterparts, based on methodologically robust studies from three different countries (Iowa Persian Gulf Study Group, 1997; Goss Gilroy, 1998; Unwin et al., 1999). Symptoms relating to cognition, the musculoskeletal system, and fatigue are more prevalent among Gulf War veterans than controls. Further, many symptoms and their clustering do not appear to fit conventional diagnoses. The conundrum is whether or not these unexplained symptoms constitute a syndrome(s) and, if so, are they best studied and treated as a unique new syndrome(s) or a variant form(s) of an existing syndrome (e.g., chronic fatigue syndrome, fibromyalgia) (see Appendix D). The very lack of definition or classification of veterans' unexplained illnesses has made it difficult to diagnose and treat many Gulf War veterans.

Additionally, the health studies reviewed in this chapter have found little or no excess mortality, hospitalizations, or birth defects in the children of veterans, although these studies have some limitations. Deployment to the Gulf War is associated with stress-related disorders, such as PTSD and depression. Yet a sizable number of veterans with unexplained symptoms do not have any psychiatric diagnoses. Further research is urgently needed to understand the nature of veterans' unexplained symptoms and their relationship to their experience in the Gulf War.

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CONTENTS

3

Methodology

This chapter discusses the methodology used by the committee in formulating its conclusions about associations between exposure to biological and chemical agents and adverse health effects. It provides information about the types of evidence the committee reviewed, how the committee assessed the strength of the evidence, and the categories of evidence the committee used to summarize its findings. Further, the chapter includes a discussion of the issues involved in the assessment of Gulf War veterans' exposures[1] to the agents of concern.

The committee has undertaken a review of the scientific and medical literature on the following agents: depleted uranium (DU), pyridostigmine bromide (PB), sarin and cyclosarin, and the anthrax and botulinum toxoid vaccines to determine whether they might be associated with adverse health effects. Although many chemical and biological agents were present during and after the Gulf War conflict, the committee chose these agents because they were of particular concern to the veterans (see Chapter 1).[2] For each agent, the committee determined—to the extent that available published scientific data permitted meaningful determinations—the strength of the evidence for associations between exposure to the putative agent and adverse health effects. Because of the general lack of exposure measurements in veterans (with some exceptions), the committee reviewed studies of other populations known to be exposed to the agents of concern. These include uranium processing workers, individuals who

[1]Exposure refers to any contact of an organism (or population) with a biological, chemical, or physical agent.

[2]Subsequent IOM studies will examine the literature on the remaining agents of concern.

may have been exposed to sarin as a result of terrorist activity, healthy volunteers (including military populations), and clinical populations (e.g., patients with myasthenia gravis treated with PB). Studying these groups allowed the committee to address the issue of whether the agents could be associated with adverse health outcomes.

In this chapter, the committee describes its approach to enable the reader to assess and interpret its findings and to assist those who may update the committee's conclusions as new information becomes available. The details of the analysis related to each agent and conclusions concerning health effects appear in subsequent chapters and in the Executive Summary. The committee's analyses have both quantitative and qualitative aspects, and reflect the evidence and the approach taken to evaluate that evidence. The methodology described in this chapter, draws from the work of previous Institute of Medicine (IOM) committees and their reports on vaccine safety (IOM, 1991, 1994a), herbicides used in Vietnam (IOM, 1994b, 1996, 1999), and indoor pollutants related to asthma (IOM, 2000). However, the conclusions in the current report depart from previous studies by distinguishing between transient and long-term health effects, and dose-related health outcomes as they are reported in the literature.

METHODS OF GATHERING AND EVALUATING THE EVIDENCE

The committee reviewed and evaluated studies from the scientific and medical literature that were identified by searches of bibliographic databases and other methods (see Appendix C). As noted, the committee did not limit its review to health effects reported by Gulf War veterans but studied all health outcomes reported in populations exposed to the agents of concern. By taking this broad and inclusive approach the committee intends to provide the Department of Veterans Affairs (VA) with a range of information about potential health outcomes for their consideration as they develop a compensation program for Gulf War veterans. Further, studies of nonveteran populations are also important for understanding those health effects with a long latency period between the time of exposure to the agent and the health effect (e.g., cancer) since long-term effects might not yet be manifest in Gulf War veterans, yet could be important for compensation decisions later in life.

The committee adopted a policy of using only peer-reviewed published literature as the basis for its conclusions. Publications that were not peer-reviewed had no evidentiary value for the committee (i.e., they were not used as evidence for arriving at the committee's conclusions about the degree of association between exposure to a particular agent and adverse health effects). The process of peer review by fellow professionals, which is one of the hallmarks of modern science, ensures high standards of quality but does not guarantee the validity or generalizability of a study. Accordingly, committee members read each article critically. In some instances, non-peer-reviewed publications provided background information for the committee and raised issues that required further

research. The committee, however, did not collect original data, nor did it perform any secondary data analysis.

In its evaluation of the peer-reviewed literature, the committee considered several important issues, including the quality and relevance of the studies; issues of error, bias, and confounding; the diverse nature of the evidence and the research; and the disparate populations being studied. Additionally, for many of the agents being studied (e.g., vaccines, PB, sarin) there were few epidemiologic studies, and much of the evidence was in the form of case studies and case reports, forms of publication that often do not provide sufficient evidence upon which to base conclusions about the statistical associations between illnesses and the agents under consideration.

TYPES OF EVIDENCE

The scientific literature on the putative agents varied from agent to agent in the number and type of published studies. For most agents, the epidemiological evidence was sparse. For only one agent was there a solid base of epidemiological studies from which the committee could draw conclusions. The extensive occupational studies of uranium workers provided a statistical foundation from which the committee could assess the strength of the association between uranium and adverse health effects. For the other agents, the committee had to rely primarily on a variety of human studies that had not been designed specifically to study adverse health effects related to the putative agents. Studies of patients with myasthenia gravis, for example, were designed primarily to examine the treatment effectiveness of PB, and had not been designed as robust epidemiologic studies (i.e., they often examined small populations or did not have control groups). The committee, however, adopted a uniform approach for evaluating the varied types of available evidence as reflected in the literature on each of the putative agents.

Animal and Other Nonhuman Studies

Studies of laboratory animals and other nonhuman systems are essential to understanding mechanisms of action, biologic plausibility, and providing information about possible health effects when experimental research in humans is not ethically or practically possible (Cohrssen and Covello, 1989; NRC, 1991). Such studies permit a potentially toxic agent to be introduced under conditions controlled by the researcher—such as dose,[3] duration, and route of exposure—to probe health effects on many body systems. Nonhuman studies are also a valuable complement to human studies of genetic susceptibility. While nonhuman

[3]The term dose is used to denote the total amount of the agent to which an organism is exposed. Dose usually implies the "exposure dose" (i.e., the total amount of the agent penetrating [or given] to an organism by a particular route of exposure) (Ballantyne, 1992).

studies often focus on one agent at a time, they more easily enable the study of chemical mixtures and their potential interactions.

Research on health effects of toxic substance includes animal studies that characterize absorption, distribution, metabolism, elimination, and excretion. Animal studies may examine acute (short-term) exposures or chronic (long-term) exposures. Animal research may focus on the mechanism of action (i.e., how the toxin exerts its deleterious effects at the cellular and molecular levels). Mechanism-of-action (or mechanistic) studies encompass a range of laboratory approaches with whole animals and in vitro systems using tissues or cells from humans or animals. Also, structure–activity relationships, in which comparisons are made between the molecular structure and chemical and physical properties of a potential toxin versus a known toxin, are an important source of hypotheses about mechanism of action.

In carrying out its charge, the committee used animal and other nonhuman studies in several ways, particularly as a marker for health effects that might be important for humans. If an agent, for example, was absorbed and deposited in specific tissues or organs (e.g., uranium deposition in bone and kidney), the committee looked especially closely for possible abnormalities at these sites in human studies.

One of the problems with animal studies, however, is the difficulty of finding animal models to study symptoms that relate to uniquely human attributes, such as cognition, purposive behavior, and the perception of pain. With the exception of fatigue, many symptoms reported by veterans (e.g., headache, muscle or joint pain) are difficult to study in standard neurotoxicological tests in animals (OTA, 1990).

For its evaluation and categorization of the degree of association between each exposure and a human health effect, however, the committee only used evidence from human studies. Nevertheless, the committee did use nonhuman studies as the basis for judgments about biologic plausibility, which is one of the criteria for establishing causation (see below).

Human Studies

Epidemiologic Studies

Epidemiology concerns itself with the relationship of various factors and conditions that determine the frequency and distribution of an infectious process, a disease, or a physiological state in human populations (Lilienfeld, 1978). Its focus on populations distinguishes it from other medical disciplines. Epidemiologic studies characterize the relationship between the agent, the environment, and the host and are useful for generating and testing hypotheses with respect to the association between exposure to an agent and health or disease. The following section describes the major types of epidemiologic studies considered by the committee.

Cohort studies. The cohort, or longitudinal, study is an epidemiologic study that follows a defined group, or cohort, over time. It can test hypotheses about whether an exposure to a specific agent is related to the development of disease and can examine multiple disease outcomes that may be associated with exposure to a given agent. A cohort study starts with people who are free of a disease (or other outcome) and classifies them according to whether or not they have been exposed to the agent under study. A cohort study compares health outcomes in individuals who have been exposed to the agent in question with those without the exposure. Such a comparison can be used to estimate a risk difference or a relative risk, two statistics that measure association. The risk difference is the rate of disease in exposed persons minus the rate in unexposed subjects. It represents the absolute number of extra cases of disease associated with the exposure. The relative risk or risk ratio is determined by dividing the rate of developing the disease in the exposed group by the rate in the nonexposed group. A relative risk greater than 1 suggests a positive association between exposure and disease onset. The higher the relative risk, the stronger is the association.

One major advantage of a cohort study is the ability of the investigator to control the classification of subjects at the beginning of the study. This classification in prospective cohort studies is not influenced by the presence of disease because the disease has yet to occur, which reduces an important source of potential bias known as selection bias (see later discussion). A cohort study design also gives the investigator the advantage of measuring and correcting another potential source of bias—confounding. As explained in the next section, when it is possible to measure a confounding factor,[4] the investigator can apply statistical methods to minimize its influence on the results. Another advantage of a cohort study is that it is possible to calculate absolute rates of disease incidence.[5] A final advantage, especially over cross-sectional studies (discussed below), is that it may be possible to adjust each subject's follow-up health status for baseline health status so that the person acts as his or her own control, that may reduce a source of variation and increase the power to detect effects. The disadvantages of cohort studies are high costs as a result of a large study population and prolonged periods of follow-up (especially if the disease is rare), attrition of study subjects, and delay in obtaining results.

A prospective cohort study selects subjects on the basis of exposure (or lack of it) and follows the cohort into the future to determine the rate at which the disease (or other health outcome) develops. A retrospective (or historical) cohort study differs from a prospective study in terms of temporal direction; the

[4]A confounding factor is a variable that is independently associated with the health outcome and may affect the results of the study because it is distributed differently in the study and control groups.

[5]Incidence is the rate of occurrence of new cases of an illness or disease in a given population during a specified period of time. Prevalence is the number of cases of an illness or disease existing in a given population at a specific point or period in time.

investigator traces back in time to classify past exposures in the cohort and then tracks the cohort forward in time to ascertain the rate of disease. Retrospective cohort studies are commonly performed in occupational health. They often focus on disease mortality rates because of the relative ease of determining vital status of individuals and the availability of death certificates to determine the cause of death.

For comparison purposes, cohort studies often use general population mortality rates (age, sex, race, time, and cause specific) because it may be difficult to identify a suitable control group of unexposed workers. The *observed* number of deaths among workers (from a specific cause such as lung cancer) is compared with the *expected* number of deaths. The expected number is calculated by taking the mortality rate in the general population and multiplying it by the number of person-years[6] of follow-up for the workers. The ratio of observed to expected deaths (which, by convention, is often multiplied by 100) produces a standardized mortality ratio (SMR). An SMR greater than 100 generally suggests an elevated risk of dying in the exposed group. Further, as discussed below many cohort studies refine their measures of health outcomes by using an internal comparison group, which may differ in exposure level but may otherwise be more similar to the cohort than the general population. Many of the studies of uranium workers are retrospective cohort studies (see Chapter 4).

The major problem with using general population rates for comparison with occupational cohorts is the "healthy-worker effect" (see Chapter 2; Monson, 1990), which arises when an employed population experiences a lower mortality rate than the general population, which consists of a mix of healthy and unhealthy people. The healthy-worker effect is usually due to lower cardiovascular and trauma deaths. A population with elevated external traumatic causes of death (e.g., Gulf War veterans), however, may be different from many occupational populations.

In calculating the SMR, the denominator (expected deaths) is derived from general population figures rather than from an otherwise comparable group of unexposed workers (which may be unavailable). The "artificially" higher denominator for expected deaths in the general population lowers the SMR, thereby underestimating the strength of the association between exposure to the agent and the cause of death. In other words, the healthy-worker effect introduces a bias that diminishes the true disease–exposure relationship.

To counter the influence of the healthy-worker effect, some studies divide the worker population into different groups, based on their levels of exposure to the agent being studied. Searching for dose–response relationships within the worker population itself is a way of reducing the potential bias introduced by the use of population controls. The problem, of course, is that measurements of dose may be imprecise or unavailable, particularly if the exposures occurred decades

[6]Person-years of follow-up refers to the sum of the observation periods for the total number of workers under study. Its purpose is to account for varying periods of employment for each study subject.

ago. Consequently, epidemiologists often rely on job classification as a surrogate means of documenting dose. Reliance on job classification introduces the possibility of misclassification bias because the classification may not be a good proxy for the actual exposure or dose. Another problem is incompleteness of records, not only in determining job classification but especially in determining whether potential confounding exposures, such as cigarette smoking by individual workers, are present. Bias, introduced by misclassification and confounding, can systematically alter study results by diluting or enhancing associations (see discussion later in this chapter).

Case-control studies. The case-control study is useful for testing hypotheses about the relationships between exposure to specific agents and disease. It is especially useful for studying the etiology of rare diseases. When health outcomes are infrequent or rare, longitudinal or cross-sectional studies must be large enough and of sufficiently long duration to accumulate enough adverse events to accurately estimate the risk of a particular agent. In case-control studies, subjects (or cases) are selected on the basis of having a disease; controls are selected on the basis of not having the disease. Cases and controls are then asked about their past exposures to specific agents. Cases and controls are matched with regard to characteristics such as age, gender, and socioeconomic status, so as to eliminate these characteristics as the cause of observed differences in past exposure. The odds of exposure to the agent among the cases are then compared with the odds of exposure among controls. The comparison generates an odds ratio,[7] a statistic that depicts the odds of having a disease among those exposed to the agent of concern relative to the odds of the disease for an unexposed comparison group. An odds ratio greater than 1 indicates that there is a potential association between exposure to the agent and the disease. The greater the odds ratio, the greater is the association. Thus, in a case-control study, subjects are selected on the basis of disease presence; prior exposure is then ascertained.

Case-control studies have the advantages of ease, speed, and relatively low cost. They are also advantageous for their ability to probe multiple exposures or risk factors. However, case-control studies are vulnerable to several types of bias, including recall bias. Other problems are identifying representative groups of cases, choosing suitable controls, and collecting comparable information about exposures on both cases and controls. These problems may lead to unidentified confounding variables that differentially influence the selection of cases or control subjects or the detection of exposure. For these reasons case-control studies are often the first, yet not the definitive, approach to testing a hypothesis.

Cross-sectional studies. In a cross-sectional study, the population of interest is surveyed at one point in time. Information is collected simultaneously about their health conditions and exposures to various agents, either present or

[7]The odds ratio is a good estimate of relative risk when the disease under study is rare.

past. The selection of individuals into the study—unlike that for cohort and case-control studies—is independent of both the exposure to the agent under study and disease characteristics. Cross-sectional studies seek to uncover potential associations between exposure to specific agents and development of disease. They may compare disease or symptom rates between groups with and without the exposure to the specific agent or may compare exposure to the specific agent between groups with and without the disease. Although cross-sectional studies need not have control groups, studies with control groups are more methodologically sound. Several health studies of Gulf War veterans are controlled cross-sectional surveys that compare a sample of veterans previously deployed to the Gulf War with a sample of veterans who served during the same period but were not deployed to the Gulf War (see Chapter 2).

Cross-sectional surveys are easy to perform and inexpensive to implement relative to cohort studies. Cross-sectional surveys can identify the prevalence of diseases and exposures in a defined population. They are useful for generating hypotheses; however, they are much less useful for determining cause-and-effect relationships, because disease and exposure data are collected simultaneously and may be self-reported (Monson, 1990). It may also be difficult to determine the temporal sequence of exposure and symptoms or disease.

Experimental Studies

Experimental studies in humans are the foremost means of establishing causal associations between exposures and human health outcomes. Experimental studies are used most frequently in the evaluation of the safety and efficacy of medications, surgical practices, biological products, vaccines, and preventive interventions. In an experiment, the investigator has control over assigning the agent to be studied and recording the outcome. Two key features of experimental studies are prospective design and use of a control group. Randomized controlled trials are considered the gold standard in experimental studies.

In randomized controlled trials, each subject has a known, often equal, probability of assignment to either the treatment or the control group. Large randomized controlled trials are designed to have all possible confounding variables occur with equal frequency in the intervention and control groups. Blinding may be another aspect of randomized controlled trials.[8] Blinding refers to shielding subjects or investigators from knowledge of whether the subjects were assigned to the treatment or the control group. Blinding is most readily accomplished when subjects in the control group receive a placebo. When both subjects and investigators are unaware of patient assignment, the study is said to be "double-blind." The objective of blinding is to reduce bias introduced by patients' and

[8]Blinding can also be part of the study design in cohort and case control studies. Disease outcome and exposures can be determined independently by different groups of researchers or the exposure assessment in case control studies can be performed by scientists who are blinded as to the disease status of the subjects.

investigators' attitudes and expectations for study outcomes. In a study of the anthrax vaccine by Brachman and colleagues (1962), workers in four goat hair processing mills were randomized to receive the vaccine or a placebo and then followed to assess the vaccine's safety and efficacy (see Chapter 7).

The value of randomized controlled trials has been so convincingly demonstrated that they are required for ensuring the safety and efficacy of all new medications introduced into the market in the United States (FDA, 1998). Estimates are that 300,000 randomized controlled trials have been carried out over the past 50 years (Randal, 1999). The main drawbacks of randomized controlled trials are their expense, the time needed for completion, and the common practice of systematically excluding many groups of patients so that the results apply to only a small fraction of potentially eligible patients.

Experimental studies are most often performed for therapeutic agents, where the only expected result is a good outcome or no effect for the subject; rarely are adverse health effects expected. Ethical considerations limit experimental studies of toxic compounds and adverse health outcomes, and guidelines for informed consent and protection of human subjects are strictly implemented (NIH, 1991).

Case Reports and Case Series

A case report is generally a detailed description of a patient's illness reported by a clinician who may suspect that the illness is the result of exposure to a specific biological or chemical agent. A case series refers to a group of patients with the same or similar disease who experienced identical or similar exposures to a specific agent. Neither case reports nor case series are formal epidemiologic studies, but both are means for generating hypotheses about exposure and disease relationships. For Gulf War veterans, registry programs established by the VA and the Department of Defense (DoD) represent a type of voluntary case series. Any veteran may come forward to receive a clinical examination and a referral for treatment (see Chapter 2). Through documentation of veterans' symptoms and diagnoses, these registries have been valuable in generating hypotheses, yet they are not designed for hypothesis testing or for establishing the prevalence of disease or specific exposures among Gulf War veterans.

The value of case reports and case series is that they can document possible associations between an environmental exposure and a particular health outcome. In some situations, they may be useful in suggesting causal relationships if the disease is rare and has a close temporal relationship to the exposure (Kramer and Lane, 1992). However, case reports and case series do not have control groups. Because case series are not population based, many cases caused by an exposure go unreported, and the prevalence of cases may be lower than in the population at large. Further, the cases may not have been caused by exposure to the specific agent (false-positive results).

CONSIDERATIONS IN ASSESSING THE STRENGTH OF THE EVIDENCE

The committee's process of reaching conclusions about the various agents and their potential for adverse health outcomes was collective and interactive. As the committee reviewed the literature on the agents under study, it took into consideration a variety of criteria (discussed below) to help evaluate the strength of the evidence for or against an association between exposure to the agent under study and adverse health outcomes. The committee assessed the evidence by considering the six general criteria (strength of association, dose–response relationship, consistency of association, temporal relationship, specificity of association, and biological plausibility) patterned after those introduced by Hill (1971). The committee also assessed the extent for potential errors in the study due to a number of factors including chance and bias (discussed later in the chapter).

Strength of Association

The strength of association is usually expressed as the magnitude of the measure of effect, for example, relative risk or odds ratio. Generally, the higher the relative risk, the stronger the association between the agent and the health effect. Moreover, the greater is the likelihood that the agent–health effect association is causal (i.e., the less likely it is to be due to undetected error, bias, or confounding). Small increases in relative risk that are consistent across a number of studies may provide evidence of an association (IOM, 1994b).

Dose–Response Relationship

A dose–response relationship refers to the finding of a greater health effect (response) with higher doses of an agent. A steep dose–response relationship strengthens the inference that an association is real. Generally, in a strong dose–response relationship, cohorts exposed to presumably low doses show only mild elevations in risk, whereas cohorts with exposure to presumably high doses show more extreme elevations in risk. However, the absence of such a relationship does not discount the possibility of an association. For example, a dose–response relationship would go undetected if the doses were all below a threshold level of exposure, beyond which the relative risk of disease increased steeply.

Many physiologic and pharmacologic actions have thresholds that result in a dose–response curve that is curvilinear rather than linear in shape. Furthermore, a particular agent may produce an effect after a brief latency or after decades (e.g., asbestos-induced mesothelioma). Other mechanisms that may alter the strictly linear dose–response curve are chemical interactions involving synergism and antagonism (e.g., a worker with significant asbestos exposure has a risk about five times greater for developing lung cancer than a person without asbestos exposure); reversibility (e.g., some toxic events are reversible as the

body has the potential for self repair); and susceptibility or resistance to a particular agent (Brooks et al., 1995).

Consistency of Association

A consistent association is similar in magnitude and direction across several studies representing different populations, locales, and times (Hill, 1965). The greater the number of studies with the same results, the more consistent is the association and the greater is the likelihood of a true association. However, consistency alone is not sufficient evidence of an association. The committee considered findings that were consistent in direction across different categories of studies to be supportive of an association. The committee did not require exactly the same magnitude of association in different populations to conclude that there was a consistent association. A consistent positive association could occur when the results of most studies were positive and the differences in measured effects were within the range expected on the basis of sampling error, selection bias, misclassification, confounding, and difference in actual dose levels (IOM, 1994b).

Temporal Relationship

The finding of an agent–disease association begins the process of trying to decide whether the agent is a cause, correlate, or consequence of the disease. Determining causality requires that exposure to the agent precede the onset of the health outcome by at least the duration of disease induction. If, in a cohort study, exposure to the agent occurs after the appearance of the health outcome, the agent could not have caused that outcome. Establishing a temporal relationship is often difficult, especially with health outcomes that have long induction periods, such as cancer. The committee interpreted the lack of an appropriate time sequence as evidence against association, but recognized that insufficient knowledge of the natural history and pathogenesis of many of the health outcomes under review limited the utility of this criterion (IOM, 1994b).

Specificity of Association

Specificity refers to the unique association between exposure to a particular agent and a health outcome (i.e., the health outcome never occurs in the absence of the agent). Two examples of highly specific associations are the pathologically distinctive tumors mesothelioma of the lung and angiosarcoma of the liver in workers exposed to asbestos and vinyl chloride, respectively. The committee recognized, however, that perfect specificity is unlikely given the multifactorial etiology of many of the health outcomes noted in this study. Additionally, the committee recognized that the agents under review might be associated with a broad spectrum of health outcomes.

Biological Plausibility

Biological plausibility reflects knowledge of the biological mechanism by which an agent can lead to a health outcome. This knowledge comes through mechanism-of-action or other studies from pharmacology, toxicology, microbiology, and physiology, among other fields, typically in studies of animals. Biological plausibility is often difficult to establish or may not be known at the time an association is first documented. The committee considered factors such as evidence in animals and humans that exposure to the agent is associated with diseases known to have similar biological mechanisms as the disease in question; evidence that certain outcomes are commonly associated with occupational or environmental exposures; and knowledge of routes of exposure, storage in the body, and excretion that would suggest the disease is more likely to occur in some organs rather than others (IOM, 1994b).

Other Considerations

It is also important to consider whether alternative explanations might account for the finding of an association. The types of studies described earlier in this chapter are often used to demonstrate associations between exposures to particular agents and health outcomes. The validity of an association, however, can be challenged by error due to chance, bias, and confounding in assembling the study populations (which are more or less representative samples from the entire relevant populations). Since these sources of error may represent alternative explanations for an observed association, they must be ruled out to the extent possible. These sources of error are important for interpreting the strength and limitations of any given study and for understanding the criteria used by the committee to evaluate the strength of the evidence for or against associations.

Chance is a type of error that can lead to an apparent association between an exposure to an agent and a health effect when none is actually present. An apparent effect of an agent on a health outcome may be the result of random variation due to sampling when assembling the study populations, rather than to the agent under study. Standard methods using confidence intervals or tests of statistical significance allow one to assess the role of chance variation due to sampling. A statistically significant finding is one in which there is little chance (usually less than 5 percent) of observing an apparent association when none really exists. A confidence interval (for a relative risk, odds ratio, or other measure of association) is centered at the estimate of the measure of interest and its range depends on the amount of variability in the sample. Although it is possible to calculate a confidence interval for any coverage probability, a 95 percent confidence interval is commonly used. If 95 percent confidence intervals were constructed for repetitions of the experiment (i.e., many different samples were drawn from the population of interest under the same circumstances), 95 percent of these intervals would contain the true value.

Bias refers to systematic or nonrandom error. Bias causes the observed value to deviate from the true value. It can weaken the association or generate a spurious association. Because all studies are susceptible to bias, a key goal is to minimize bias or to adjust the observed value of the association using special methods to correct for bias. There are three general sources of error that may compromise the results of an investigation, including selection bias, confounding, and information bias.

Selection bias can occur in the recruitment of study subjects to a cohort when the study and control groups differ from each other by a factor that is likely to affect the results. Thus, the observed cohort differs from the population at large by some unmeasured variable that could predict the outcome. Non-population-based cross-sectional studies are particularly vulnerable to selection bias.

Confounding occurs when a variable or characteristic can account for part or all of an apparent association. For example, if inhaled uranium particles appear to be associated with the development of lung cancer, cigarette smoking may confound this outcome if the cohort exposed to uranium had more members that smoked than the unexposed cohort. Confounding variables can be either measured or unmeasured. With measured confounders, carefully applied statistical adjustments can control for or reduce their influence. With unmeasured confounders, no adjustment is possible. With studies of uranium miners, for example, it is usually not possible to adjust for the role of cigarette smoking by individuals, since the employee records of decades ago seldom contained information about smoking.

Information bias results from the way in which the data are collected, for example, from measurement errors, imprecise measurement, and misdiagnosis. These types of errors may be uniform across the entire study population or may affect some parts of the population more than others. Bias may result from misclassification of study subjects with respect to the outcome variable. Other common sources of information bias are due to the inability of study subjects to accurately recall the circumstances of the exposure (recall bias) or to the likelihood that one group more frequently reports what it remembers than another group (reporting bias). Information bias is especially pernicious when it affects one comparison group more than another.

SUMMARY OF THE EVIDENCE

As seen below in the discussion of categories of association, the committee distinguishes between "sufficient evidence of a causal relationship" and "sufficient evidence of an association." Thus, before describing the categories used to summarize its findings, the committee provides a brief discussion of the concepts of causation and association.

Understanding Causation and Association

A principal objective of epidemiology is to understand whether exposures to specific agents are associated with disease or other health outcomes and, with additional available information, to decide whether such associations are causal. Although they are frequently used synonymously, the terms "association" and "causation" have distinct meanings.

Epidemiologic studies can establish statistical associations between exposure to specific agents and health effects. In the types of epidemiologic studies described earlier in this chapter, the degree of an association is often measured by relative risks, odds ratios, and SMRs. Epidemiologic studies find different degrees of association, depending on the magnitude of the relative risk, odds ratio, or SMR, and its variability and on the ability to exclude or reduce sources of error. To conclude that an association exists, it is necessary for an agent to occur together with the health outcome more frequently than expected by chance alone. Further, it is almost always necessary to find that the effect occurs consistently in several studies. Epidemiologists seldom consider one study, taken alone, sufficient to establish an association; rather, it is necessary to replicate the findings in other studies in order to draw conclusions about the association. Results from separate studies sometimes conflict with one another. It is sometimes possible to attribute discordant study results to characteristics such as the soundness of study design, the quality of execution, and the influence of different forms of error and bias. Studies that result in a statistically significant measure of association account for the role of chance in producing the observed result. When the measure of association does not show a statistically significant effect, it is important to consider the size of the sample and whether the study had the power to detect a rare but important effect.

Study designs differ in their ability to provide a valid estimate of an association (Ellwood, 1998). Randomized controlled trials are the most robust type of evidence, whereas cohort or case-control studies are more susceptible to chance, bias, and confounding. Case series and case reports carry the least weight, but may be the only information available, especially for an extremely rare event (e.g., a hypersensitivity reaction). For most of the agents reviewed in this report, the committee had to rely on case series and case reports because more robust epidemiologic studies were not available.

Determining whether a given statistical association rises to the level of causation requires inference (Hill, 1971). In order to infer a causal association, one must bring together evidence from different studies and apply well-established criteria that have been refined over more than a century (Hill, 1971; Evans, 1976; Wegman et al., 1997). The criteria for inferring a causal relationship are strength of association, dose–response relationship, consistency of association, temporal relationship, specificity of association, and biological plausibility (as discussed above). Strictly speaking, assessing causality was not within the charge of this committee, but the criteria for causality were helpful as the com-

mittee evaluated the strength of the evidence for or against associations between health effects and exposure to the agents being studied.

Categories of Association

The committee used five previously established categories to classify the evidence for association between exposure to a specific agent and a health outcome. The categories closely resemble those used by several IOM committees that evaluated vaccine safety (IOM, 1991, 1994a), herbicides used in Vietnam (IOM, 1994b, 1996, 1999), and indoor pollutants related to asthma (IOM, 2000). Although the categories imply a statistical association, the committee had sufficient epidemiologic evidence to examine statistical associations for only one of the agents under study (i.e., depleted uranium), there was very limited epidemiologic evidence for the other agents examined (i.e., sarin, pyridostigmine bromide, and anthrax and botulinum toxoid vaccines). Thus, the committee based its conclusions on the strength and coherence of the data in the available studies. In many cases, these data distinguished differences between transient and long-term health outcomes related to the dose of the agent. Based on the literature, it became incumbent on the committee to similarly specify the differences between dose levels and the nature of the health outcomes. This approach led the committee to reach conclusions about long- and short-term health effects, as well as health outcomes related to the dose of the putative agents. The final conclusions expressed in Chapters 4–7 represent the committee's collective judgment. The committee endeavored to express its judgments as clearly and precisely as the available data allowed. The committee used the established categories of association from previous IOM studies, because they have gained wide acceptance for more than a decade by Congress, government agencies, researchers, and veteran groups.

- *Sufficient Evidence of a Causal Relationship.* Evidence is sufficient to conclude that a causal relationship exists between the exposure to a specific agent and a health outcome in humans. The evidence fulfills the criteria for sufficient evidence of an association (below) and satisfies several of the criteria used to assess causality: strength of association, dose–response relationship, consistency of association, temporal relationship, specificity of association, and biological plausibility.
- *Sufficient Evidence of an Association.* Evidence is sufficient to conclude that there is a positive association. That is, a positive association has been observed between an exposure to a specific agent and a health outcome in human studies in which chance, bias, and confounding could be ruled out with reasonable confidence.
- *Limited/Suggestive Evidence of an Association.* Evidence is suggestive of an association between exposure to a specific agent and a health outcome in humans, but is limited because chance, bias, and confounding could not be ruled out with confidence.

- *Inadequate/Insufficient Evidence to Determine Whether an Association Does or Does Not Exist*. The available studies are of insufficient quality, consistency, or statistical power to permit a conclusion regarding the presence or absence of an association between an exposure to a specific agent and a health outcome in humans.
- *Limited/Suggestive Evidence of No Association.* There are several adequate studies, covering the full range of levels of exposure that humans are known to encounter, that are mutually consistent in not showing a positive association between exposure to a specific agent and a health outcome at any level of exposure. A conclusion of no association is inevitably limited to the conditions, levels of exposure, and length of observation covered by the available studies. In addition, the possibility of a very small elevation in risk at the levels of exposure studied can never be excluded.

These five categories cover different degrees or levels of association, with the highest level being sufficient evidence of a causal relationship between exposure to a specific agent and a health outcome. The criteria for each category incorporate key points discussed earlier in this chapter. A recurring theme is that an association is more likely to be valid if it is possible to reduce or eliminate common sources of error in making inferences: chance, bias, and confounding. Accordingly, the criteria for each category express varying degrees of confidence based upon the extent to which it has been possible to exclude these sources of error. To infer a causal relationship from a body of evidence, the committee relied on long-standing criteria for assessing causation in epidemiology (Hill, 1971; Evans, 1976).

COMMENTS ON INCREASED RISK OF ADVERSE HEALTH OUTCOMES AMONG GULF WAR VETERANS

As discussed in the beginning of this chapter, the committee reviewed the available scientific evidence in the peer-reviewed literature in order to draw conclusions about associations between the agents of interest and adverse health effects in all populations. The committee placed its conclusions in categories that reflect the strength of the evidence for an association between exposure to the agent and health outcomes. The committee could not measure the likelihood that Gulf War veterans' health problems are associated with or caused by these agents. To address this issue, the committee would need to compare the rates of health effects in Gulf War veterans exposed to the putative agents with the rates of those who were not exposed, which would require information about the agents to which individual veterans were exposed and their doses. However, as discussed throughout this report, there is a paucity of data regarding the actual agents and doses to which individual Gulf War veterans were exposed. Further, to answer questions about increased risk of illnesses in Gulf War veterans, it would also be important to know the degree to which any other differences be-

tween exposed and unexposed veterans could influence the rates of health outcomes. This information is also lacking for the Gulf War veteran population. Indeed most of the evidence that the committee used to form its conclusions about the association of the putative agents and health effects comes from studies of populations exposed to these agents in occupational and clinical settings, rather than from studies of Gulf War veterans. Due to the lack of exposure data on veterans, the committee could not extrapolate from the level of exposure in the studies that it reviewed to the level of exposure in Gulf War veterans. Thus, the committee could not determine the likelihood of increased risk of adverse health outcomes among Gulf War veterans due to exposure to the agents examined in this report.

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CONTENTS

4

Depleted Uranium

Uranium is a naturally occurring element that is present in soil (with an average concentration of 3 parts per million), rocks, surface and underground water, air, plants, and animals (ATSDR, 1999b). As a result, it occurs in trace amounts in many foods and in drinking water. An individual's daily intake of uranium is estimated to be 1–2 micrograms (μg) in food and 1.5 μg in each liter of water consumed (ATSDR, 1999b). The International Commission on Radiological Protection (ICRP) has reported that the average uranium content of the human body is 90 μg, with 69 μg in the skeleton and 7 μg in the kidneys (ICRP, 1975). A range in total body content of uranium of 2–62 μg has been noted in human postmortem studies (NRC, 1988). The primary civilian use of uranium is as fuel for nuclear power plants. Additionally, minute amounts are used in the production of ceramic glazes, light bulbs, and photographic chemicals (ATSDR, 1999b).

Natural uranium is a radioactive element with three principal isotopes: ^{234}U, ^{235}U, and ^{238}U. These isotopes are alpha particle emitters. Alpha particles are positively charged ions composed of two protons and two neutrons. Due to their size and charge, alpha particles lose their kinetic energy quickly and have little penetrating power. The range of an alpha particle is approximately 4 cm in air and considerably less (25–80 μm) in tissue (ATSDR, 1999a). As a result, pure uranium is principally an internal radiation hazard. Uranium isotopes decay to other radioactive elements that eventually decay to stable isotopes of lead (ATSDR, 1999b). In the decay process, beta and gamma radiation[1] are emitted.

[1]Beta particles are high-energy electrons; the path length of a beta particle averages 0–15 m in air and 0–1 cm in solids. Gamma radiation is an external radiation hazard be-

The isotopes of uranium have the same chemical properties because they all have the same number of protons, 92. However, variation in the number of neutrons gives the isotopes different radiological properties. The radioactivity of isotopes can be compared using specific activity, a measurement of the number of nuclear transformations (disintegrations) per second per unit mass (Box 4.1). The most abundant naturally occurring uranium isotope, ^{238}U, has the lowest specific activity (0.33 microcuries per gram [μCi/g]) (U.S. AEPI, 1995). The high specific activity of ^{234}U (6,200 μCi/g) contributes to more than half of the radioactivity of natural uranium even though by weight its percentage is extremely small (Table 4.1). Enriched uranium is quantified by its percentage of ^{235}U (specific activity 2.2 μCi/g) which can range from 2 percent to more than 90 percent (U.S. AEPI, 1995). Because of the high percentage of ^{238}U in natural uranium (Table 4.1) and that isotope's low specific activity, natural uranium is considered a low-level radioactive element.

Uranium is also categorized as a heavy metal (i.e., any metal with a specific gravity of 5.0 or greater). The chemical toxicity of a uranium compound varies depending on the nature of the compound, its solubility, and its route of exposure.

There are a number of radiological protection regulations and guidelines. The U.S. Nuclear Regulatory Commission's (U.S. NRC's) regulations for occupational dose to individual adults state an annual limit of the total effective dose equivalent of 5 rem per year (50 millisieverts [mSv] per year) (10 CFR 20). For members of the general public, the U.S. NRC's regulations require that the total effective dose equivalent to individual members of the general public not exceed 0.1 rem in a year (1 mSv), exclusive of the dose contributions of background radiation (10 CFR 20).

Depleted uranium (DU) is a by-product of the enrichment process used to make reactor-grade uranium. Because of the different percentages of uranium isotopes in depleted uranium (Table 4.1), its specific activity (14.8 mBq/μg) is

TABLE 4.1 Percentage of Uranium Isotopes by Weight

Isotope	Natural Uranium	Depleted Uranium[a]
^{238}U	99.2745	99.745
^{235}U	0.7200	0.250
^{234}U	0.0055	0.005

[a]Depleted uranium may have trace amounts of ^{236}U (U.S. AEPI, 1995).

SOURCES: Durakovic, 1999; Lide, 1999.

cause it is highly penetrating. Gamma radiation (high-energy photons) is the energy released due to the change in the energy state of the nucleus.

BOX 4.1
Units of Measurement

Specific Activity

The **curie** (Ci) is the traditional unit of measure of nuclear transformations (disintegrations) per second of unit mass. It is a concentration defined as the ratio of the amount of radioactivity divided by the mass or volume of radioactive substance. The International System unit for specific activity is the **becquerel** (Bq).

Absorbed Dose

The **gray** (Gy), formerly the rad, is the unit that describes the magnitude of absorbed radiation in terms of energy deposited on a tissue. However, the amount of energy deposited in tissue does not account for differences in the biological effects of different radiation types.

Dose Equivalent

The **rem** (Roentgen-equivalent-man) is the traditional unit of measure that incorporates the relative biological damage caused by different radiation types and deposition mechanisms. The International System unit for the biologically effective dose, dose equivalent, is the **sievert**, (Sv).

	Specific Activity	Absorbed Dose	Biologically Effective Dose
Units	curie (Ci) becquerel (Bq)	gray (Gy) rad (old standard unit)	rem sievert (Sv)
Conversion	1 Bq = 1 transformation or disintegration per second = 2.7 x 10^{-11} Ci	1 Gy = 100 rad	1 mSv = 0.001 Sv 1 Sv = 100 rem

SOURCE: ATSDR, 1999a,b.

approximately 40 percent lower than that of naturally occurring uranium (25.4 mBq/μg) and considerably lower than that of enriched uranium (approximately 1,750 mBq/μg) (Harley et al., 1999). However as discussed above, the chemical properties of depleted uranium are the same as those of the enriched and natural forms.

The chemical and physical properties of depleted uranium are ideal for many military and commercial uses. It is 65 percent more dense than lead (with a density of 18.9 g/cm^3), has a high melting point (2070°F, 1132°C), is highly pyrophoric (it ignites when it fragments), has a tensile strength comparable to most steels, and is chemically highly reactive (Kirk, 1981). The density of DU and its ability to self-sharpen are properties that attracted the attention of the Department of Defense (DoD) beginning in the late 1950s, as the military

sought to increase the armor penetration of munitions (OSAGWI, 1998). In addition, depleted uranium is useful as a ballast or counterweight in aircraft and gyroscopes because it lends itself to casting into small but dense weights. Additional uses of depleted uranium include as radiation shielding and as a chemical catalyst (it is a strong reducing agent) (Kirk, 1981; Lide, 1999).

The process of converting uranium ore into enriched uranium, with depleted uranium as a by-product, begins with the mining of uranium-containing ore (work previously conducted in deep underground mines but now mostly in surface mines). Sites of significant uranium deposits include the western United States, Canada, southern Africa, and Australia. The milling process crushes the ore and then leaches uranium from the ore with sulfuric acid or alkaline carbonates (du Preez, 1989). The dissolved uranium precipitates as triuranium octaoxide, U_3O_8 (termed "yellowcake"); this process does not alter the ratio of radioisotopes of uranium (U.S. AEPI, 1995). The enrichment process converts uranium to its hexafluoride (UF_6) form, which is a gas, and separates the various isotopes using gaseous diffusion or centrifuge technology, thereby increasing the percentage of ^{235}U in UF_6. The remainder of the UF_6 (depleted UF_6) has a smaller proportion of both ^{235}U and ^{234}U relative to the enriched UF_6. The final steps of the milling process are the reduction of depleted UF_6 to uranium tetrafluoride ("green salt") which is further reduced to depleted uranium metal. The Nuclear Regulatory Commission defines depleted uranium as uranium with less than 0.711 percent ^{235}U by weight (10 CFR 40.4). Department of Defense specifications state that depleted uranium used by DoD must have a ^{235}U concentration of less than 0.3 percent (U.S. AEPI, 1995).

In the Gulf War, weapons systems utilized depleted uranium (frequently alloyed three-fourths of 1 percent with titanium by weight to reduce oxidation) for offensive and defensive purposes (Parkhurst et al., 1995; OSAGWI, 1998). Heavy armor tanks had a layer of DU armor to increase protection. Offensively, depleted uranium increases the penetration effectiveness of the kinetic energy cartridges and ammunition rounds used by the Army (105- and 120-mm tank ammunition), Air Force (armor piercing munitions for the Gatling gun mounted on the A-10 aircraft), Marine Corps (Harrier aircraft and tank munitions), and Navy (rounds for the Phalanx Close-in Weapon System)[2] (OSAGWI, 1998). The Army used an estimated 9,500 depleted uranium tank rounds during the Gulf War, many as training and practice rounds (OSAGWI, 1998).

Known exposure of U.S. personnel to depleted uranium during the Gulf War occurred as the result of friendly fire incidents, cleanup operations, and accidents (including fires). DU-containing projectiles struck 21 Army combat vehicles (15 Bradley Fighting Vehicles and 6 Abrams tanks) (U.S. AEPI, 1995). Additionally, U.S. forces used DU rounds to destroy three unoccupied Abrams tanks in order to prevent them from being captured by the enemy, and five

[2]The only firings reported during the Gulf War of this weapon system were test firings and an accidental discharge. The Navy is transitioning to tungsten rounds (OSAGWI, 1998).

Abrams tanks became contaminated when DU rounds were involved in onboard fires (U.S. AEPI, 1995). After the war, assessment teams and cleanup and recovery personnel (including Explosive Ordnance Disposal, Battle Damage Assessment, and Radiation Control teams and salvage personnel) may have had contact with DU-contaminated vehicles or depleted uranium munitions. In July 1991, a large fire occurred in Camp Doha near Kuwait City. This site housed a number of combat-ready vehicles, and the series of blasts and fires damaged or destroyed vehicles and munitions including M1A1 tanks and depleted uranium munitions. Troops at the scene and those involved in cleanup efforts may have been exposed to DU residue. Other troops may have been exposed through contact with vehicles or inhalation of DU-containing dust (Fahey, 2000).

In estimating the number of U.S. personnel exposed to depleted uranium and the extent of this exposure, the DoD Office of the Special Assistant for Gulf War Illnesses (OSAGWI) categorized potential DU exposure scenarios into three levels (OSAGWI, 1998).

Level I exposure, the highest level, occurred in or near combat vehicles when they were struck by DU rounds or when soldiers entered vehicles soon after the impact. An estimated 143–173 people may have experienced Level I exposure through wounds caused by DU fragments, inhalation of airborne DU particles, ingestion of DU residues, or wound contamination by DU residues. Some Gulf War veterans, including those with internal DU fragments, are participating in the Depleted Uranium Follow-up Program, a medical surveillance follow-up study, at the Baltimore VA Medical Center (McDiarmid et al., 2000). There are ongoing efforts to expand this program to include additional veterans.

Level II, the intermediate exposure level, occurred when soldiers and civilian employees worked on DU-contaminated vehicles or were involved in cleanup efforts from the Camp Doha fire. More than 700 individuals may have had Level II exposure through inhalation of dust containing DU particles and residue, or ingestion from hand-to-mouth contact or contamination of clothing.

Level III, the lowest level of exposure, occurred when troops were downwind from burning DU ammunition, DU-contaminated vehicles, or the Camp Doha fire or when personnel entered DU-contaminated Iraqi tanks. These Level III exposures could have occurred though inhalation or ingestion. Hundreds of people are thought to have experienced potential Level III exposure, but there is little to substantiate these estimates (OSAGWI, 1998).

Since the Gulf War, there has been extensive modeling and testing of potential depleted uranium exposure, including evaluation of radiological and chemical hazards and characterization of DU aerosols (Parkhurst et al., 1991, 1995; Parkhurst and Scherpelz, 1994; GAO, 2000). It has been estimated that the exposure (Level I) of individuals (excluding those with embedded DU fragments), who were inside an Abrams M1A1 tank when a single DU penetrator entered the crew compartment, would be approximately 0.48 rem for a 15-minute exposure (OSAGWI, 1998). The U.S. Army Center for Health Promotion and Preventive Medicine (CHPPM) is in the process of completing a com-

prehensive exposure assessment that includes quantitative risk assessments for selected health end points.

This chapter examines the published scientific literature on potential health effects of uranium and depleted uranium. The chapter begins with an overview of the toxicology and animal studies and then examines the scientific literature on human health effects, most of which comes from epidemiologic studies of workers exposed to uranium and from human case reports. In summarizing the scientific research on the toxicology of uranium, the committee frequently references the Agency for Toxic Substances and Disease Registry's (ATSDR's) *Toxicological Profile for Uranium* (ATSDR, 1999b). ATSDR's extensive report is a review and assessment of the peer-reviewed literature on the toxicological end points. The ATSDR report was reviewed by a nongovernmental panel and by scientists from federal agencies.

TOXICOLOGY

As discussed above, uranium is both a heavy metal and a low-specific-activity radioactive element. Studies on the toxicity of uranium have examined both its chemical and its radiological effects. The primary routes of exposure to uranium for humans are through ingestion or inhalation; the effects of dermal exposure and embedded fragments have also been studied.

The amount of uranium that the body absorbs depends largely on the route of exposure and the solubility of the uranium compounds to which the individual is exposed. Insoluble uranium compounds may remain within the pulmonary tissues, especially the pulmonary lymph nodes, for a long time and thus constitute a localized radiological hazard. As a general rule, uranium is less readily absorbed from the intestinal tract than from the respiratory tract, resulting in lower doses per unit intake. Chemical toxicity, characterized predominantly by renal dysfunction as a consequence of exposure to soluble uranium, and lung injury potentially caused by the ionizing radiation from uranium decay isotopes are the best-characterized consequences of exposure to uranium compounds. However, the chemical and radiological properties of uranium could act cooperatively to cause tissue damage, and therefore, it cannot be assumed that excess cancers would be due solely to the radiological effects of uranium or that organ damage is exclusively due to its heavy-metal properties.

Pharmacokinetics and Toxicokinetics

Absorption

Inhalation exposure. The site of deposition of uranium particles in the respiratory tract is the result of a combination of physical forces that govern particle behavior in an air stream, as well as the anatomy of the respiratory tract (Gordon and Amdur, 1991). The site of deposition affects the degree of ura-

TABLE 4.2 Dissolution Types of Uranium Compounds

Type F (fast)	Type M (medium)	Type S (slow)
Uranium hexafluoride (UF_6)	Uranium tetrafluoride (UF_4)	Uranium dioxide (UO_2)
Uranium tetrachloride (UCl_4)	Uranium trioxide (UO_3)	Triuranium octaoxide (U_3O_8)
Uranyl fluoride (UO_2F_2)		
Uranyl nitrate hexahydrate [$UO_2(NO_3)_2 \cdot 6H_20$]		

nium absorption, the clearance mechanisms that are available to remove uranium particles, and the severity of the consequences of tissue damage to the respiratory system.

Inhaled uranium dust particle deposition in the various regions of the respiratory tract and lung (extrathoracic, tracheobronchial, and deep pulmonary or alveolar) depends on the particle's aerodynamic diameter. An aerodynamic diameter is typically assigned to particles that are nonspherical in shape and incorporates both the density and the diameter of the particle, as well as its aerodynamic drag. It represents the particle as the diameter of a unit-density sphere having the same terminal velocity as the particle, whatever its size, shape, or density (Gordon and Amdur, 1991). Larger particles are deposited in the tracheobronchial region; mucociliary action then transports the particles to the pharynx where they are swallowed. Smaller particles reach the terminal bronchioles and the alveoli. The ICRP has developed extensive models of the dosimetry of inhaled radioactive materials (ICRP, 1994).

At the alveolar level, the more soluble uranium compounds (categorized as Type F for *fast* dissolution [e.g., UF_6 and uranyl nitrate hexahydrate]) are taken up within days by the systemic circulation (Table 4.2). The less soluble uranium compounds (Type M for *medium* dissolution [e.g., uranium tetrafluoride, UF_4, and uranium trioxide, UO_3]) are more likely to remain for weeks in the pulmonary tissue and associated lymph nodes. The relatively insoluble compounds (categorized as Type S for *slow* dissolution [e.g., uranium dioxide, UO_2, and triuranium octaoxide U_3O_8]) are least likely to enter the systemic circulation and may remain for up to several years within the lung and tracheobronchial lymph nodes (ATSDR, 1999b). The lungs and the tracheobronchial lymph nodes are the two major sites of accumulation for Type S uranium compounds (administered as UO_2) in dogs, monkeys, and rats, accounting for greater than 90 percent of the total body burden of uranium after inhalation of those compounds (Leach et al., 1970).

Given their high density, most occupationally inhaled uranium particle-containing dusts have an aerodynamic diameter that does not permit them to be carried to the peripheral part of the lung (Berlin and Rudell, 1986; Morris et al., 1992). Estimates based on measurements in uranium processing plants (Davies, 1961), suggest that only between 1 and 5 percent of uranium particle-containing

dusts will penetrate the lungs. The rest will deposit in the upper respiratory tract and eventually will be swallowed and go through the gastrointestinal tract.

Oral exposure. The absorption of uranium across the gastrointestinal tract generally increases with increasing solubility of the compound. Absorption is greatest for the relatively soluble uranium compounds. Notably, even with the soluble compounds, only a small fraction of uranium is absorbed across the gastrointestinal epithelium. Humans ingesting uranyl nitrate hexahydrate or uranyl nitrate absorb only 0.5–5 percent of the ingested dose (Hursh et al., 1969; Karpas et al., 1998). Adult hamsters that received actinide preparations of uranyl nitrate and uranium dioxide through an intragastric tube absorbed 0.77 and 0.11 percent of the total doses, respectively (Harrison and Stather, 1981). After administering UO_2 to rats by the same route, other investigators could not detect any uranium in liver, kidney, muscle, bone, brain, blood, and urine (Lang and Raunemaa, 1991).

Dermal absorption. Dermal absorption of uranium compounds in humans has not been characterized (ATSDR, 1999b). In animals, the soluble uranium compounds uranyl nitrate hexahydrate (0.5–7 g/kg body weight) and ammonium uranyl tricarbonate (7 g/kg body weight) penetrated the skin of experimental rats within 15 minutes of application (de Rey et al., 1983). Forty-eight hours after exposure, uranium was no longer present on the skin, and the rats had experienced severe toxic signs ranging from weight loss to death, indicating absorption of uranium into the systemic circulation. No penetration of uranium through the skin occurred after applying the more insoluble compound uranium dioxide (de Rey et al., 1983). Other uranium compounds, such as uranium tetrafluoride, uranium tetrachloride, and uranium trioxide, are absorbed through the skin of mice, rats, and guinea pigs (Orcutt, 1949). Although the absorption rate was relatively low (0.1 percent of uranium applied to the skin), the amount of absorbed uranium was sufficiently high to cause toxicity (Orcutt, 1949). These animal studies show that percutaneous absorption is an effective route for soluble uranium compounds to enter the systemic circulation. However, the application of these findings to human dermal exposure is unclear because the concentrations of uranium that were applied to the skin were extremely high.

Transport and Biotransformation

Once absorbed, uranium forms soluble complexes with bicarbonate, citrate, or proteins in the plasma (Dounce and Flagg, 1949; Stevens et al., 1980; Cooper et al., 1982). Approximately 47 percent of blood uranium forms a complex with bicarbonate in plasma, 32 percent of uranium binds to plasma proteins, and 20 percent binds to erythrocytes (Chevari and Likhner, 1968).

Distribution

Inhalation of less soluble uranium compounds (Type M and Type S) is associated with retention of uranium in bronchial lymph nodes as well as lung tissue itself (Leach et al., 1970), from which it passes slowly into the systemic circulation. Of the total uranium absorbed into the circulation, 85 percent deposits in the bone (Donoghue et al., 1972). Of the remaining uranium (15 percent), greater than 90 percent is distributed to the kidneys; detectable amounts are also present in the liver. In bone, uranium replaces calcium in the hydroxyapatite complex. The half-life of uranium in bone is approximately 300 days (Harley et al., 1999). In the kidney, uranium accumulates primarily in the proximal tubule.

Excretion and Retention

Systemic clearance. The stability of the bicarbonate complex of uranium depends on the pH of the solution and differs in various bodily compartments (Berlin and Rudell, 1986). The low-molecular-weight bicarbonate complex passes through the renal glomerulus and is excreted in the urine at a rate that depends on urinary pH. At high pH, small amounts of uranium are retained within the walls of the tubular lumen of the kidney. At low pH, bicarbonate–uranyl (and citrate–uranyl) complexes dissociate (Bassett et al., 1948). The uranyl ion forms complexes with proteins on the surface of cells lining the tubule, a process that may account for uranium-induced tubular dysfunction (see below). In contrast to the low-molecular-weight uranyl–bicarbonate complex, uranium that is protein bound is more likely to remain in blood since little protein passes through the glomerulus.

In humans, approximately two-thirds of an intravenous injection of uranium is eliminated from the plasma within 6 minutes, and 99 percent of the uranium is eliminated from the plasma 20 hours after injection (Struxness et al., 1956; Luessenhop et al., 1958). The kidneys excrete more than 90 percent of hexavalent soluble uranium salt injected intravenously, and less than 1 percent is excreted in the feces. Approximately 70 percent of the dose is excreted within the first 24 hours, followed by a slower phase with a half-time exceeding several months (Bassett et al., 1948).

Inhalation exposure. The rate of deposition and clearance of uranium-containing particles from the lung depends on their chemical form and particle size. As discussed above, mucociliary action transports most of the larger particles from the respiratory system to the pharynx, where they are swallowed and then eliminated in the feces. The clearance of the smaller particles that are deposited in the lungs depends on the solubility of the compounds. Particles that contain the more soluble forms of uranium are more rapidly absorbed into the bloodstream and excreted in urine. For example, in studies of rat lung retention of uranium administered as an aerosol powder (commercial yellowcake) with median aerosol concentrations from 0.04 to 0.34 μg U/L (micrograms of ura-

nium per liter) (Damon et al., 1984), the half-time for Type F uranium compounds was between 1 and 5 days. The body retains Type S compounds for much longer. The half-time of uranium deposited in the lungs of dogs, rats, and monkeys exposed up to 5 years to uranium dioxide dust (5.1 mg U/m^3) was approximately 15 months (Leach et al., 1973).

Reports from human subjects occupationally exposed to insoluble uranium compounds suggest a two-phase clearance process, consisting of a short phase with a biological half-time between 11 and 100 days and a slow phase of clearance with a biological half-time between 120 and 1,500 days (Hursh and Spoor, 1973). The biological half-time of uranium dioxide in the lungs of occupationally exposed workers was estimated to be 109 days in one study (Schieferdecker et al., 1985).

The aerosol by-products of exploded DU munitions are primarily the uranium oxides—uranium trioxide, triuranium octaoxide, and uranium dioxide (OSAGWI, 1998). Uranium trioxide behaves more like a soluble uranyl salt than the insoluble oxides (U_3O_8 and UO_2) and is rapidly removed from the lung (half-time, 4.7 days). More than 20 percent of the exposure burden of UO_3 passes into the systemic circulation, and approximately 20 percent of the excreted uranium appears in the urine (Morrow et al., 1972). Conversion of UO_3 to uranyl hydroxide hydrate followed by cation exchange with structural hydroxyl groups is a possible mechanism for the high solubility of UO_3 in biological fluids (Stuart et al., 1979). Uranium dioxide and triuranium octaoxide have slow dissolution rates (Type S dissolution), and the mechanical processes (mucociliary transport) and particle size determine their pulmonary clearance rates.

Oral exposure. The low rate of gastrointestinal absorption of uranium in humans results in approximately 95 percent of ingested uranium being eliminated in feces without being absorbed; the remainder is excreted in urine (Wrenn et al., 1985; Spencer et al., 1990). The average gastrointestinal uptake of uranium in adult humans is estimated at 1.0–1.5 percent (Leggett and Harrison, 1995). Although differences in uranium uptake with age have not been reported, more definitive information is needed for children (Leggett and Harrison, 1995).

Animal studies indicate that uranium absorption through the gastrointestinal tract depends strongly on its chemical form when ingested and the length of time between the last meal and the ingestion of uranium. Both rats and rabbits absorb about 0.06 percent of ingested uranium in the gastrointestinal tract (Tracy et al., 1992). The distribution and retention of uranium in the skeleton and kidneys of rats are comparable to parameters reported for humans. Studies with rats indicate that the majority of ingested uranium (99 percent) is eliminated in the feces without being cycled through the bile. Most of the uranium absorbed through the gastrointestinal tract is excreted within a few days in urine, with a half-time of 2–6 days (Durbin and Wrenn, 1975).

Depleted uranium fragments. Pellmar and colleagues (1999a) studied the organ distribution of uranium dissolved from DU fragments. The study examined rats that had DU and/or tantalum pellets surgically implanted within the gastrocnemius muscle. Tantalum, an inert metal that is widely used in prosthetic devices,

was used as a control. Tissue samples were collected at day 1, and at 6, 12, and 18 months after implantation. Bone and kidney were the primary reservoir for the uranium that had dissolved from embedded DU fragments. Dissolved uranium also localized within the central nervous system (CNS), lymph nodes, testes, and spleen (Pellmar et al., 1999a). Low levels of uranium were noted in the serum at all time points. The size of the pellets diminished with time.

Animal and In Vitro Studies

The following section summarizes and highlights a number of key animal and in vitro studies of the toxic effects of uranium. Researchers have examined the health effects of exposure to uranium via the inhalation, oral, and dermal routes; there are also studies on the effects of injected uranium and embedded DU fragments. A more thorough description of these studies and others can be found in the recent ATSDR (1999b) review.

Nonmalignant Respiratory Effects

Acute exposure to uranium (UF_6; 10-minute exposure at 637 mg U/m^3) resulted in gasping and severe irritation of the nasal passages in rats and mice (Spiegl, 1949); nasal hemorrhage occurred in rats after 5-minute exposure to 54,503 mg/m^3 (Leach et al., 1984). These effects were most likely due to the hydrolysis of UF_6 to hydrofluoric acid, a potent toxicant to respiratory tract epithelium (Spiegl, 1949; Leach et al., 1984).

Rats, mice, and guinea pigs exposed to uranium hexafluoride for an intermediate duration (6 hours a day for 30 days at 13 mg U/m^3) showed pulmonary edema, hemorrhage, emphysema, and inflammation of the bronchi and alveoli (Spiegl, 1949). Cats and dogs exposed for 30 days to 18 mg U/m^3 as uranium tetrafluoride or 5 weeks exposure to 9.2 mg U/m^3 as uranyl fluoride exhibited rhinitis (Dygert et al., 1949). Notably, uranium dioxide and triuranium octaoxide, insoluble uranium compounds, did not lead to pulmonary toxicity. Hemorrhagic lungs were noted in dogs exposed to carnotite uranium ore, likely reflecting deeper penetration of this material into the dogs' respiratory tract compared to guinea pigs and mice, which remained asymptomatic in similar exposure studies.

Rats, rabbits, guinea pigs, and dogs exposed to aerosols containing 0.05–10 mg U/m^3 of various uranium compounds for 7–13 months did not suffer uranium-related histological damage to the lungs (Cross et al., 1981a,b). In a comprehensive study by Leach and colleagues (1970), lung damage did not occur in rats and dogs exposed to 5 mg U/m^3 as uranium dioxide dust for 1–5 years (5.4 hours a day, 5 days a week). Occasional patchy hyaline fibrosis was evident in the tracheobronchial lymph nodes of dogs and monkeys exposed for a minimum of 3 years to the same concentrations of uranium (Leach et al., 1970).

Carcinogenic and Genotoxic Effects

Filippova and colleagues (1978) studied the carcinogenic effects of intratracheal injection with 90 percent enriched ^{235}U as tetravalent uranium (0.57–18.7 mg U/kg body weight) or hexavalent uranium (0.55–5.32 mg U/kg body weight) in the rat. A variety of cancers developed in both groups of uranium-injected rats (osteosarcoma, carcinoma of the lungs and kidneys, reticulolymphosarcoma of the lung, and leukemia) at a rate that was statistically significantly different from controls (24 percent with tetravalent uranium, 24 percent with pentavalent uranium, and 12 percent in controls). Cross and colleagues (1981a) measured the effects of inhalation exposure on tumor development in golden Syrian hamsters. Animals inhaled uranium ore dust at a concentration of 19 mg U/m^3 for 16 months. The authors reported no apparent increase in the number of tumors in several tissues (liver, kidney, spleen, trachea, lungs, and heart) compared to unexposed animals.

In a chronic inhalation study, Leach and colleagues (1973) reported that uranium dioxide exposure (5 mg U/m^3) led to pulmonary lymphatic neoplasm development and atypical epithelial proliferation in 30–46 percent of exposed beagle dogs. Although the rate of tumor development was 50–100 times higher than the expected spontaneous incidence in this species, the authors cautioned against extrapolating these findings to humans, given the infrequent occurrence of these types of lymphatic neoplasms in humans. Long-term feeding studies found no evidence of cancer in several animal species that were exposed to high levels of uranium (Maynard and Hodge, 1949; ATSDR, 1999b). The committee did not locate studies on the tumorigenic effects of uranium following dermal exposure.

A recent report is the first to suggest that, at least in vitro, DU can cause human cell transformation to a neoplastic phenotype, an effect that is comparable to other biologically reactive and carcinogenic heavy-metal compounds, such as nickel (Miller et al., 1998a). DU uranyl chloride-transformed cells displayed anchorage-independent growth, tumor formation in nude mice, expression of high levels of the *k-ras* oncogene, reduced production of the Rb tumor-suppressor protein, and elevated levels of sister chromatid exchanges per cell; all are associated with carcinogenic processes.

To assess the potential mutagenic effects of long-term exposure to internalized depleted uranium, Sprague-Dawley rats received 20 pellets of either tantalum or DU in various combinations (low DU: 4 DU and 16 tantalum pellets; medium DU: 10 DU and 10 tantalum pellets; high DU: 16 DU and 4 tantalum pellets) (Miller et al., 1998b). The rats excreted significant concentrations of uranium in urine throughout the 18 months of the study (224 ± 32 μg U/L urine in the low-dose rats and 1010 ± 87 μg U/L urine in the high-dose rats at 12 months). Investigators assessed the mutagenic potential of uranium at 0 days, 6 months, 12 months, and 18 months after implantation. Urine from animals implanted with DU pellets at each of the assessed time points enhanced mutagenic activity in *Salmonella typhimurium* strain TA98 and the Ames II mixed strains (TA7001–7006). Urine samples from animals implanted with tantalum alone

(and in the absence of detectable uranium levels in the urine) did not enhance mutagenic activity in these strains. In DU-implanted animals, urine mutagenicity increased in a dose- and time-dependent manner, demonstrating a strong positive correlation with urine uranium levels. In contrast to urine samples, the sera of animals implanted with either DU or tantalum pellets did not enhance mutagenicity in any bacterial strain. This study indicates that the mutagenic potential of uranium increases as its urinary concentration increases. Given that serum samples did not contain increased levels of uranium or show mutagenic activity in the same tests, it is possible that the strong positive relationship between urinary uranium and mutagenicity is causally related to the presence of uranium in the urine. The authors have speculated that uranium, possibly complexed with various ligands found in urine (carbonates, proteins, minerals, phospholipids), caused the increased mutagenic potential (Miller et al., 1998b).

Nonmalignant Renal Effects

In animal studies, uranium has been shown to have low-level metallotoxic effects on the renal system (reviewed in Stopps and Todd, 1982; Gilman et al., 1998a,b,c; ATSDR, 1999b). Other heavy metals (e.g., lead, arsenic, mercury) are much more toxic at the same dose level (ATSDR, 1999b). In general, renal injury occurs within days of exposure and manifests itself as a change in the proximal convoluted tubules, resulting in increased urinary enzyme excretion (alkaline phosphatase, lactate dehydrogenase, and leucine aminopeptidase). Hyaline casts, or casts containing necrotic cells shed from the tubular epithelium, are present at all levels of the tubular system (Berlin and Rudell, 1986). Parallel with tubular damage, glomerular changes also occur, principally in the basement membranes of glomerular capillaries. The corresponding functional changes in the kidney are proteinuria, impaired *p*-aminohippurate (PAH) clearance, increased clearance of amino acids and glucose, and decreased sodium reabsorption. After severe damage, renal inulin and creatinine clearance decreases (Stopps and Todd, 1982). Generally, if the uranium dose is sublethal, regeneration of the damaged epithelium commences within 2–3 days or when exposure ceases (Stopps and Todd, 1982; Berlin and Rudell, 1986; Gilman et al., 1998b; ATSDR, 1999b). Tolerance to uranium has also been observed after exposure to sublethal doses. In a 5-year study in dogs and monkeys of inhaled dust containing insoluble uranium dioxide, kidney injury did not occur at exposure levels of 5 mg U/m^3 (Leach et al., 1970). Following a 91-day exposure to uranyl nitrate hexahydrate in drinking water (0.96, 4.8, 24, 120, or 600 mg/L), histopathological lesions were observed in the kidneys of male and female New Zealand white rabbits in all groups including the lowest-exposure groups (Gilman et al., 1998c). Pathological changes included lesions of tubular epithelial cells (apical nuclear displacement and vesiculation, cytoplasmic vacuolation, and dilation), glomeruli (capsular sclerosis), and renal interstitium (reticulin sclerosis and lymphoid cuffing). Studies of dermal and ocular absorption of UO_3

in rabbits indicate that uranium was sufficiently well absorbed to cause kidney damage and even death from renal failure (Voegtlin and Hodge, 1949).

Several mechanisms may account for uranium-induced kidney damage. A mechanism involving bicarbonate activity in the kidney has been postulated. Uranium combines with bicarbonate, citrate, or plasma proteins in blood. At low pH, the bicarbonate–uranyl, and citrate–uranyl, complexes split (Bassett et al., 1948), and the resulting uranyl ion may combine with proteins on the tubular wall to cause renal damage. A second possible mechanism suggests that uranium compounds inhibit both sodium-dependent and sodium-independent adenosine triphosphate (ATP) utilization and mitochondrial oxidative phosphorylation in renal tubules (Brady et al., 1989).

Nonmalignant Neurological Effects

Neurological effects following inhalation of uranium occur in cats and dogs. In a 30-day inhalation study, dogs exposed to 0.5–18 mg U/m^3 (as UF_6 gas) exhibited muscular weakness and instability of gait on day 13 at the highest concentration tested (Dygert et al., 1949). Cats exposed by inhalation to 18 mg U/m^3 (as UF_6 gas) had similar symptoms, beginning on day 7 (Dygert et al., 1949). Exposure to high concentrations of uranium (9.5 mg U/m^3 as uranyl nitrate hexahydrate for 30 days) has been associated with anorexia in dogs (Roberts, 1949). A similar effect was noted at the highest concentration tested in both dogs and cats exposed to uranyl fluoride (0.15–9.2 mg U/m^3; 5 weeks) (Dygert et al., 1949). Acute cholinergic toxicity of uranium in rats occurred after a single oral dose of uranyl acetate dihydrate (11–717 mg U/kg) (Domingo et al., 1987). Exposure-related signs of neurotoxicity were not apparent in rats exposed to uranyl nitrate hexahydrate for 91 days at levels up to 600 mg/L drinking water, which is equivalent to a time-weighted average equivalent dose of 37 and 54 mg U/kg body weight per day for male and female rats, respectively (Gilman et al., 1998a).

There is limited evidence that large doses of uranium can cause changes in the central nervous system of animals. Purjesz and colleagues (1930) and Verne (1931) detailed epithelial degeneration of the choroid plexi in the CNS of dogs and rabbits exposed to toxic doses of soluble uranium salts. Verne (1931) described CNS changes that appeared relatively late (just prior to death) after very large injections of uranium. CNS changes were noted primarily in the cerebral and cerebellar cortices, and in the latter they were confined to pyramidal and Purkinje cells. Purjesz and colleagues (1930) studied the effects of uranium nitrate in dogs. After repeated delivery of the compound (1–4 g), the animals generally lived for only a few days. In each case, morphological changes were apparent in the choroid plexus. As the doses in these studies are orders of magnitude greater than human exposure to uranium, it is difficult to determine the relevance of these findings to humans.

The chronic long-term health consequences of exposure to DU fragments have been addressed by Pellmar and colleagues (1999a). As described earlier,

rats were surgically implanted with sterilized DU and/or tantalum pellets within the gastrocnemius muscle (low dose DU: 4 DU and 16 tantalum pellets; medium dose: 10 DU and 10 tantalum pellets; high dose: 16 DU and 4 tantalum pellets). The purpose of these experiments was to establish an animal model that would provide insight into the injuries sustained by Gulf War veterans from embedded DU fragments and would make it possible to evaluate the biological effects of intramuscularly embedded DU fragments. Many of these DU fragments were not removed from the veterans because the surgical procedure would have produced extensive tissue damage.

As early as 1 month after pellet implantation and at subsequent sample times (6 months), brain concentrations of uranium were statistically elevated in DU-implanted rats compared to controls implanted with tantalum (the high-dose group received 20 DU pellets). At 18 months, several brain areas were independently assessed. Uranium levels increased in a dose-dependent manner with the number of DU pellets implanted. The levels of uranium were not uniform throughout the brain; uranium levels in the motor cortex, frontal cortex, midbrain, and vermis were elevated in DU-implanted rats compared to tantalum-implanted controls for both the medium- and the high-dose groups. The animals were not perfused prior to sacrifice, and the authors did not account for the uranium bound to red blood cells. Nonetheless, given the relatively low serum levels of uranium in the same animals (8.09 ng [nanograms] U/ml serum in the high-dose animals at 18 months), blood trapped within blood vessels does not account for the reported brain levels of uranium (~125 ng U/g for the same group). This study is the first to suggest that in the rat animal model, uranium can accumulate within the central nervous system. The mechanism of uranium transport into the CNS is unknown.

A follow-up study by the same group (Pellmar et al., 1999b) assessed the potential for electrophysiological changes in the hippocampus of rats implanted with DU fragments. At 12 months, the amplitudes of synaptic potentials were significantly greater in tissues derived from high-dose (20 pellets) DU-implanted rats compared with controls (tantalum implanted). In the same animal model, uranium did not affect locomotor activity, discrimination learning, or the results of a battery of general functional measures (Pellmar et al., 1997). The abnormal electrophysiological measurements were not apparent 18 months after exposure to 20 pellets of DU (high dose). The authors suggest that by 18 months the effects of aging and DU exposure converge, thereby obscuring the effects of the metal. The lack of an effect of DU on locomotor activity, discrimination learning, and a general functional observation battery makes it difficult to interpret the significance of uranium accumulation in the brain. It is possible that the behavioral measures of performance in learning tasks may have been insufficiently sensitive to detect the effects of brain uranium. No nephrotoxicity occurred in these animals (at urinary uranium concentrations of 1,009 ± 87 ng/ml 12 months after exposure to uranium). The study suggests that retained embedded DU fragments are associated with increased brain uranium concentrations.

Gastrointestinal Effects

No gastrointestinal effects occurred in animals administered unenriched uranium nitrate orally in doses as high as 664 mg/kg per day for up to 2 years (Maynard and Hodge, 1949). A study of rats exposed to uranyl nitrate hexahydrate in drinking water (up to 40 mg/kg per day) for 28 days found no treatment-related histopathological changes (Gilman et al., 1998a). No gastrointestinal effects occurred in rabbits exposed for 91 days to uranyl nitrate hexahydrate in drinking water (0.96, 4.8, 24, 120, or 600 mg/L) (Gilman et al., 1998b,c).

Hepatotoxicity

Uranium-induced hepatotoxicity has not been a prominent finding in most animal studies (ATSDR, 1999b). No changes occurred in the liver of dogs administered insoluble uranium acetate dihydrate for 30 days at doses as high as 7,859 mg U/kg per day (Maynard and Hodge, 1949). Inhalation exposure to 17 mg U_3O_8/m^3 for 26 days or to 22 mg UO_2/m^3 for 30 days had no hepatic effects (Dygert et al., 1949). Similarly, animals exposed to 10 mg UO_3/m^3 by inhalation for up to 2 years showed no changes in hepatic function (Stokinger et al., 1953).

Reproductive and Developmental Effects

A small number of studies addressed the effects of uranium on fertility, general reproductive parameters, or offspring survival. Llobet and colleagues (1991) evaluated the fertility of uranium-treated Swiss mice. Male mice received high doses of uranyl acetate dihydrate (10, 20, 40, and 80 mg/kg per day) in drinking water for 64 days and were then mated with untreated females. There was a significant but non-dose-related decrease in the pregnancy rate of these animals. Body weights were significantly depressed only in the group of adult male mice treated with 80 mg/kg per day. Testicular function and spermatogenesis were not affected by uranium at any dose, as evidenced by normal testes and epididymis weights and normal spermatogenesis. Vacuolization of Leydig cells was seen in the high-dose (80 mg/kg per day) group. These data indicate that normal dietary intake of uranium does not cause any adverse effect on testicular function in mice, with a safety factor of more than 1,000.

Paternain and colleagues (1989) studied the effect of uranyl acetate dihydrate on reproduction, gestation, and postnatal survival in Swiss mice. Male mice received oral uranyl acetate dihydrate (5, 10, and 25 mg/kg per day) for 60 days prior to mating with female mice treated orally (at the same doses) for 14 days prior to mating. Oral administration of the uranium compound to the female mice continued throughout mating, gestation, parturition, and nursing of the litters. Lethal effects on the embryo occurred only in the high-dose (25 mg/kg per day) group. The number of dead young per litter increased significantly at birth and at day 4 of lactation in that group. The growth rate in offspring was always significantly lower for uranium-treated animals. However,

uranium failed to cause any adverse effects on fertility, general reproductive parameters, or offspring survival (ATSDR, 1999b). No studies have reported chemical or radiological effects of uranium on fetal development after inhalation or dermal exposure to uranium compounds for any duration.

Other Health Effects

Cardiovascular effects. Animal studies report no adverse effects on cardiovascular function subsequent to oral or inhalation exposure to uranium (Dygert et al., 1949; Maynard and Hodge, 1949). Filippova and colleagues (1978) instilled 90 percent ^{235}U-enriched soluble tetravalent and uranyl nitrate hexahydrate salts into the trachea of rats and found blood vessel dystrophy and enlargement of the heart. Rabbits exposed for 91 days to uranyl nitrate in drinking water (0.96, 4.8, 24, 120, or 600 mg/L) did not suffer cardiovascular effects (Gilman et al., 1998b,c). Cardiovascular effects also did not occur in rats exposed to 0.2 mg U/m^3 as uranium hexafluoride for 1 year (Stokinger et al., 1953) or in rats, mice, guinea pigs, and rabbits exposed to 4.8 mg U/m^3 triuranium octaoxide for 26 days (Dygert et al., 1949).

Dermal effects. Inhalation and oral exposures to uranium compounds have not led to dermal effects in animals (Spiegl, 1949). Dermal application of uranium compounds was associated with mild skin irritation, severe dermal ulcers, or superficial coagulation necrosis and inflammation of the epidermis in rabbits (Orcutt, 1949). An applied dose of 237 mg U/kg as uranyl nitrate hexahydrate on the skin of rats resulted in swollen and vacuolated epidermal cells and damage to hair follicles and sebaceous glands (de Rey et al., 1983). In rats exposed to U_3O_8 (0.012 g per day) in 30 daily topical applications, the epidermis was thinner than in control animals, and skin permeability was higher (Ubios et al., 1997).

Ocular effects. In animals, encrusted eyes and conjunctivitis occurred after inhalation of 13 mg U/m^3 (UF_6) for 30 days (Spiegl, 1949) as well as with uranium tetrachloride (Dygert et al., 1949). The ocular effects were caused by direct contact of the eye with uranium aerosol or vapor.

Musculoskeletal effects. The effects of inhaled uranium on the musculoskeletal system of animals have not been examined. There were no histopathologic findings in rat or rabbit muscles after exposure to orally administered uranyl nitrate in drinking water (up to 40 mg U/kg per day for 28 day, or up to 53 mg U/kg per day for 91 days in Sprague-Dawley rats; or up to 53 mg U/kg per day for 91 days in rabbits). However, acute uranium intoxication (2 mg/kg of body weight) with [^{238}U]uranyl nitrate has been shown to inhibit bone formation, an effect believed to be due to the direct action of uranium on bone-forming cells or their precursors (Gugliemotti et al., 1985; Ubios et al., 1998).

Hematological effects. Inhalation exposure to ammonium diurnate ($[NH_4]_2U_2O_7$) dust (6.8 mg U/m^3, 6 hours per day, 30 days) caused a decrease in red blood cell count and hemoglobin concentration in rats (Dygert et al., 1949). Inhalation exposure to uranyl nitrate hexahydrate (9.5 mg U/m^3, 8 hours per day,

30 exposure days) caused a reduction in erythrocyte numbers and hemoglobin levels (Roberts, 1949). Significant increases in myeloblasts and lymphoid cells of the bone marrow occurred after inhalation exposure to uranium peroxide or uranium trioxide (15.4 mg U/m^3, 5 hours per day, 23 days, and 16 mg U/m^3, 6 hours per day, 24 days, respectively) (Dygert et al., 1949). However, a series of intermediate-duration inhalation studies showed no adverse effects of uranium on the blood (ATSDR, 1999b). Similarly, the majority of animal studies show no adverse effects of orally administered uranium compounds on blood. For example, in New Zealand white rabbits exposed for 91 days to uranium (uranyl nitrate hexahydrate) in drinking water (0.96, 4.8, 24, 120, or 600 mg U/L), hematological and biochemical parameters did not change (Gilman et al., 1998a,b,c).

EPIDEMIOLOGIC STUDIES: DESCRIPTION OF THE STUDIES

General Considerations

This section contains descriptions of epidemiologic studies of the human health effects of exposure to uranium. The section begins with an overview of the studies of uranium miners. The studies in this cohort have limited relevance to the depleted uranium exposures of Gulf War veterans because, as described below, the primary disease-causing exposures for the miners were not to uranium, but to radon. The remainder of the section provides detailed descriptions of studies on workers occupationally exposed to uranium in uranium-processing plants. The results of these studies appear in the next section of the chapter.

Although depleted uranium is the form of uranium that was present in the Gulf War theater, there are only a few studies of its health effects. Therefore, the committee relied on studies of the health effects of natural and processed uranium. As noted earlier in the chapter, the chemical characteristics of an element are independent of its isotopic form. As a result, DU has the same chemical effects as naturally occurring or enriched uranium. Given the same dose by the same route, the health effects that are due to the chemical characteristics of uranium should be identical from natural, enriched, or depleted uranium exposures. Thus, studies of the chemical effects of natural and processed uranium will provide a good indication of what studies of DU would show.

The literature examining the health effects of exposure to ionizing radiation is extensive. Recent reports including those by the NRC (National Research Council) and ATSDR (NRC, 1988, 1990, 1999; ATSDR 1999a) summarize this work. High-dose human and animal studies have shown that radiation is carcinogenic and that the incidence of cancer increases with the dose of radiation (ATSDR, 1999a). The principal isotope in natural and depleted uranium, ^{238}U, has a long half life (4.5 billion years) and primarily decays by alpha particle emission. Alpha particles have a very short range and little penetrating power. They are therefore a hazard to humans only in close proximity to human tissue.

For these reasons, natural uranium is considered a low-level *internal* radiation hazard. Thus, the focus of the discussion on radiation in this chapter is on the effects of radiation from uranium in the body, rather than from external radiation. Since the specific activity (a measure of radioactivity) of depleted uranium is 40 percent lower than that of natural uranium (and much lower than enriched uranium), any health effects that are a consequence of the radioactive nature of uranium would be expected to be less prevalent in people exposed to DU than in people exposed by the same route to the same amount by weight of natural or enriched uranium.

Studies of Uranium Miners

The committee examined studies of health effects in uranium miners, but concluded that these studies have limited relevance because the primary disease-causing exposures were not to uranium but to radon decay products. The principal form of radiation exposure of uranium miners in underground mines has been to inhalation of alpha particles emitted by radon decay products in poorly ventilated mines (NRC, 1999). Radon progeny are known to increase the risk of lung cancer (NRC, 1999). In addition, miners were exposed to other possibly toxic dusts and, potentially, to diesel gas fumes, which might cause cancer and other diseases of the lung (NRC, 1999). Another serious limitation of most studies of uranium miners is the lack of information on cigarette smoking. The experience of uranium miners with diseases other than those of the respiratory tract could inform our knowledge of the consequences of uranium mining in organ systems other than lung. However, the literature on uranium miners has focused largely on lung and other cancers, and most publications have used mortality rather than morbidity as the outcome measure.

Radon is a radioactive decay product of uranium. In a confined, poorly ventilated area, such as a mine shaft, radon gas diffuses from the surrounding uranium-containing rock and accumulates in the atmosphere within the mine shaft, where miners inhale it. Radon progeny (polonium-218, lead-214, bismuth-214, and polonium-214) decay rapidly (with half-lives of 30 minutes or less) by emitting alpha particles. These isotopes attach to dust particles, are inhaled and deposited on the bronchial epithelium, and decay before natural clearance mechanisms can remove them. However, in relatively well-ventilated work spaces, such as uranium mills or uranium fabricating plants, radon gas is present in low concentrations. Furthermore, some uranium refining processes remove radium, the immediate parent of radon in the uranium decay series. Exposure to radon decay products is known to be associated with increased risk of lung cancer (NRC, 1999).

The Committee on the Biological Effects of Ionizing Radiation (BEIR) of the National Research Council has extensively studied the published literature on the health effects of radon and other internally deposited alpha-particle emitters such as uranium (NRC, 1988, 1990, 1999). The following section briefly summarizes some of the literature on health effects in uranium mine workers.

A series of studies describes the mortality experiences of uranium miners in the Colorado Plateau states (Colorado, Utah, New Mexico, and Arizona) of the United States (Lundin et al., 1969; Saccomanno et al., 1971, 1976, 1986; Archer et al., 1973b, 1976; Auerbach et al., 1978; Band et al., 1980; Whittemore and McMillan, 1983; Hornung and Meinhardt, 1987; Roscoe, 1997). In an early report in the series, Lundin and colleagues (1969) examined mortality among 3,414 white underground uranium miners from 1950 to 1967. Total mortality increased approximately 50 percent above the rates expected for white males of the same geographic area; this excess was largely accounted for by violent deaths and cancers of the respiratory system. Most of the excess respiratory cancer deaths occurred 10 or more years after the individual's first uranium mining experience. Overall, the risk of respiratory cancer increased with cumulative estimated exposure to radon progeny during mining. Apart from the increases in respiratory cancer and violent deaths, the only other statistically significant finding was a reduced risk of death from major cardiovascular–renal disease among uranium miners, consistent with the expected healthy-worker effect (see later discussion). Many other publications in this series have focused on lung cancer or its premalignant precursors.

A recent update of the mortality of this cohort examined vital status through December 31, 1990 (Roscoe, 1997). The study found increased mortality risks in this cohort of 3,238 white male uranium miners for lung cancer (SMR = 580, 95% confidence interval [95% CI] 520–640). The study found a significant exposure–response trend for lung cancer with increased exposure to radon progeny and for duration of employment in uranium mining. Deaths from chronic nephritis were not significantly elevated in this update.

A case-control analysis of the Colorado Plateau miners examined lung cancer cases in a population of 9,817 miners during a 20-year period from 1960 to 1980 (Saccomanno et al., 1986). The study matched 489 cases of death from lung cancer with 992 non-cases to control for age and smoking history. There was a strong positive association between uranium mining and risk of lung cancer; the relative risk for those with 11 or more years of underground mining work was 8.5. Mining and cigarette smoking interacted multiplicatively in the multivariate model of the predictors of lung cancer.

Several studies have focused specifical1y on uranium mining and risk of lung cancer among Navajo men (Gottlieb and Husen, 1982; Samet et al., 1984). Samet and colleagues conducted a population-based case-control study using the New Mexico tumor registry to identify 32 cases of lung cancer occurring among Navajo men between 1969 and 1982. The authors identified two controls for each case. Of the 32 Navajo men with lung cancer, 72 percent (23) had been employed as uranium miners, whereas none of the controls had been miners. Another study followed the vital status from 1960 to 1990 of a cohort of 757 Navajo uranium miners who worked in the Colorado Plateau (Roscoe et al., 1995). Elevated SMRs were found for lung cancer (330, 95% CI 230–460), tuberculosis (260), and chronic lung disease (260). SMRs were reduced for heart disease, circulatory

disease, and liver cirrhosis. The SMR for lymphatic or hematopoietic disease was 80 (3 observed cases compared with 3.7 expected cases).

The relation between lung cancer mortality and uranium mining was also the subject of several reports from Canada (Muller et al., 1985; Nair et al., 1985; Howe et al., 1986; Kusiak et al., 1993). In a cohort of 8,487 workers employed between 1948 and 1980 in a uranium mine in Saskatchewan, a total of 65 lung cancer deaths occurred (Howe et al., 1986). There was a highly significant linear relationship between the estimated level of exposure to radiation from radon daughters and risk of lung cancer. Nair and colleagues (1985) provided additional data on other causes of death in this population. In addition to lung cancer, there were significant excess death rates for trauma, which in part reflected the safety hazards of mining. They also provided mortality data for 2,332 miners who worked in the Port Radium facility in the Northwest Territories. As with the Saskatchewan miners, mortality rates for lung cancer and trauma were significantly increased. For bone cancer, there were no cases, compared with 0.60 expected; there were 17 cases of lymphoma compared with 15.7 expected.

A study by Muller and colleagues (1985) examined the mortality experience of many types of miners (e.g., nickel, copper, gold, uranium) in Ontario between 1955 and 1986. In the cohort of uranium miners, 121 lung cancer deaths were identified; 70.54 were expected based on the general male population of Ontario. Risk increased with exposure to radiation from radon daughters, and the risk was greatest at an interval between 5 and 14 years after exposure. This study also provided data for other causes of death in this population. Deaths from silicosis and chronic interstitial pneumonia were significantly increased (11 versus 2.14 expected) as were traumatic deaths. There were 26 deaths from lymphatic and hematopoietic malignancies versus 27.4 expected; there were 2 deaths from bone cancer compared with 1.38 expected deaths.

Studies of uranium miners in Czechoslovakia provide further information on mortality from cancers other than lung cancer and from other diseases (Tomasek et al., 1993, 1994). The area near the Czech and German border has been a site for mining for many centuries, and lung disease has been a disproportionate cause of death since the sixteenth century (Tomasek et al., 1994). Tomasek and colleagues (1993, 1994) followed the mortality experience of 4,320 male mine workers from 1948–1959 to the end of 1990. Lung cancer mortality was greatly elevated in this population (SMR = 508, 95% CI 471–547), but mortality from all other cancers combined was not (SMR = 111, 95% CI 98–124) (Tomasek et al., 1993). Other than lung cancer, significant increases in cancer mortality were seen only for liver cancer (SMR = 167) and cancer of the gallbladder and extrahepatic bile ducts (SMR = 226). They found only 2 deaths from bone cancer (SMR = 69) and 11 deaths from lymphoma (SMR = 109). Despite exposures to high levels of radon, arsenic, and dust, mortality from chronic respiratory disease was only slightly and nonsignificantly elevated (SMR = 121). The observed deaths from urinary diseases in this cohort were lower than expected (SMR = 77). In addition to lung cancer, mortality was greater than expected for accidents, homicide, mental disorders, cirrhosis, and nonrheumatic circulatory disease (Tomasek et al., 1994).

In summary, the studies of uranium miners consistently show a large increase in deaths from lung cancer. However, these studies are of limited relevance to veterans of the Gulf War because the miners were exposed to high concentrations of radon gas as a consequence of working underground in poorly ventilated confined spaces. Radon decays into short-lived alpha-particle-emitting isotopes that have been found to increase the risk of lung cancer (NRC, 1999). Although long-lived isotopes of uranium, plutonium, and thorium are also present in lung tissue and thoracic lymph nodes of uranium miners (Singh et al., 1983, 1986, 1987, 1989), it has not been possible to distinguish their contribution to disease from that of radon progeny.

Studies of Uranium Processing Workers

The most important sources of evidence about the human health effects of exposure to uranium are studies of people who worked in plants whose purpose was to process and refine raw uranium ore into ^{235}U-enriched uranium metal for use in weapons and nuclear reactors (Table 4.3). Their exposure history differs from that of uranium miners because they worked in an environment that had little radon gas, an exposure that confounds any attempt to link uranium exposure in mine workers to effects on the lung, an important potential site for disease caused by uranium. The principal exposure of uranium processing workers is to uranium oxides and derivative uranium compounds produced during the uranium refinement process. It is important to note, however, that uranium is not the sole potential hazard in the industrial settings described below. Studies vary in the nature of the work being conducted and the extent to which workers were exposed to enriched uranium, soluble and insoluble uranium compounds, other radioactive elements (e.g., radium, thorium), and other potentially hazardous industrial chemicals (e.g., sulfuric acid, fluorocarbons).

Occupational studies involve exposure that occurred in the course of day-to-day tasks, rather than as a single large exposure. The exposure was greatest in the early days of the uranium processing and machining industry; as the industry adopted improved safety measures, the degree of exposure decreased. The principal route of entry for uranium in occupational exposures was inhalation of dusts to which uranium compounds were attached. In this sense, the exposure was similar in character, albeit far more prolonged, to that of Gulf War veterans involved in cleanup actions after friendly fire incidents. The exposure in the case of uranium processing plant workers occurred over a period of months to many years, in contrast to the much shorter period of exposure in veterans involved in cleanup actions.

This section is a study-by-study description of the epidemiologic studies of uranium-processing workers and includes information on the exposure measures (where available) and the analytic methods used in each study. This section does not contain any results. Results appear in the following section, which is a disease-by-disease analysis of the strength of the evidence for associations between health outcomes and exposure to uranium.

Uranium Mill Workers on the Colorado Plateau (Wagoner et al., 1964; Archer et al.,1973a; Waxweiler et al., 1983)

In addition to the many studies of Colorado Plateau uranium miners, the mortality experience of uranium mill workers in the Colorado Plateau states has been a focus of study. The process of enriching uranium ore during the milling process leads to the exposure of workers to many substances, except during the final stage of purification. Airborne dust in the mills can contain vanadium, thorium, and radium isotopes in addition to the primary compound, uranium. Additionally, in the early years, substantial silica levels were present in uranium mills (Waxweiler et al., 1983).

The earliest study by the U.S. Public Health Service (Wagoner et al., 1964) examined cancer mortality in a prospectively identified cohort of uranium miners and millers. One subcohort of 611 white male millers had no reported mining exposure. The men volunteered for at least one physical examination. The authors ascertained vital status as of December 31, 1962, and compared mortality rates with the male population of the Colorado Plateau states.

A study by Archer and colleagues (1973a) continued the follow-up of the mill worker cohort with some additions through 1967. The study examined 662 white men who worked in uranium mills in 1950–1953 and were available for medical examinations in 1950, 1951, and 1953. Investigators obtained a thorough occupational history of this prospectively defined cohort. Social Security Administration (SSA) records and many other sources were used to trace the vital status of all but 1 percent of the cohort. Comparisons were made to the mortality experience of all white men who lived in the Colorado Plateau states.

The third study was a retrospective cohort analysis examining the mortality through 1977 of uranium mill workers on the Colorado Plateau (Waxweiler et al., 1983). The study identified 2,002 men who worked at least 1 year in a mill before 1972 and who had not worked in uranium mines; the study cohort may have overlapped with that of the previous two studies, but the extent of overlap is unclear. Deaths between 1940 and 1977 were identified using Social Security records; expected deaths were based on rates in the entire U.S. population.

Nuclear Fuels Fabrication Plant Workers (Hadjimichael et al., 1983)

United Nuclear Corporation fabricated nuclear fuels from enriched uranium in a plant in Connecticut. The company asked the Yale University Department of Epidemiology and Public Health to investigate possible effects of low-level exposure to radiation and other industrial nonradiation exposures in the manufacturing plant (Hadjimichael et al., 1983). Manufacturing processes involved receiving enriched uranium, machining the uranium into appropriate shapes, encapsulating it with a metal covering, and assembling the product into larger components for use as reactor fuel. The report states that approximately one-fifth of the employees had received occupational whole-body exposure to

TABLE 4.3 Epidemiologic Studies of Uranium Processing Workers

Reference	Study Design	Description	Radiation Dose in Exposed Subjects	Study Group (*n*)
Wagoner et al., 1964	Cohort	Prospective cohort study of mortality experience of white male uranium millers and miners in the Colorado Plateau states	Not known	611 millers
Archer et al., 1973a	Cohort	Prospective cohort study of mortality experience of white male uranium millers in the Colorado Plateau states	Not known	662
Polednak and Frome, 1981	Cohort	Mortality experience of white male employees (1943–1947) at a uranium conversion and enrichment plant in Oak Ridge, TN	Not known	18,869
Hadjimichael et al., 1983	Cohort	Mortality and cancer incidence experience of employees (1956–1978) in a nuclear fuel fabrication plant in Connecticut	0.5% had ≥ 10 rem cumulative dose[a]	4,106
Waxweiler et al., 1983	Cohort	Mortality experience of male uranium millers in the Colorado Plateau states	Not known	2,002
Stayner et al., 1985	Cohort	Mortality experience of workers at a phosphate fertilizer production facility in Florida	Not known	3,199
Brown and Bloom, 1987	Cohort	Mortality experience of white male uranium enrichment workers in Ohio	Not known	5,773

Dupree et al., 1987	Cohort	Mortality experience of white male employees of a uranium processing facility in Buffalo, NY	37.9% had >10 rem/year estimated dose of internal radiation to the lung[b]	995
Checkoway et al., 1988	Cohort	Mortality experience of white male employees (1947–1979) at a nuclear materials fabrication plant, in Oak Ridge, TN	29% had ≥ 10 rem cumulative internal radiation dose[c]	6,781
Frome et al., 1990	Cohort	Mortality experience of white male workers at Oak Ridge uranium enrichment and laboratory facilities	Not known	28,008
Dupree et al., 1995	Case control	Cases of lung cancer at four uranium processing operations	5% of cases had ≥ 0.5 cGy cumulative internal radiation dose	787
Ritz, 1999	Cohort	Mortality experience of white male employees at a uranium processing plant in Ohio	8.2% had ≥ 10 rem internal radiation dose[b]	4,014

[a]Percentage of those with known exposure (of the total cohort of 4,106 individuals, exposure was known for 786 individuals; 4 individuals had ≥ 10 rem).
[b]Data in the study were given in units of millisieverts and have been converted to rems.
[c]Percentage of those with known exposure (of the total cohort of 6,781 individuals, exposure was known for 3,490).

gamma and x-ray radiation, and approximately 10 percent were at risk of internal alpha-particle exposure through inhalation or possibly ingestion.

The study cohort of 4,106 included all employees who worked at the plant for at least 6 months between 1956 and 1978. The authors constructed a list of 16 groups of job titles based on the similarity of the industrial radiation exposure within each group. Two groups had little exposure. Employment records had sufficient detail to permit the authors to assign each patient to the job category in which he or she had worked the longest. Thus, the data on each employee included one job category and the length of time in this category. SSA records and mortality records of the Connecticut Department of Health Services provided the vital status of each employee. Death certificates were available for 93.1 percent of the deceased. The vital status of 5.7 percent of the employees was unknown at the end of the study in 1978. By matching employee names to names in the Connecticut Tumor Registry, the authors measured the incidence of cancer.

Film badges, worn by all employees who worked in areas with exposure to radiation, provided a measure of cumulative external radiation. The plant monitored internal radiation by measuring urinary uranium excretion. Starting in 1969, employees with high urine uranium levels also received in vivo counting of lung radioactivity. Therefore, information on internal exposure levels was not complete for all employees. The basis of comparison of death rates was the Connecticut population. The authors determined each person's years at risk by dividing the time from entering employment to the end of the study into 5-year intervals and summing the risk of disease in each interval over all intervals. They also performed a multivariate analysis with death from a disease as the dependent variable and year of employment, smoking, cumulative radiation exposure, previous occupational radiation exposure, and whether the person was an industrial worker or an office worker as independent variables.

Phosphate Fertilizer Production Workers (Stayner et al., 1985)

Stayner and colleagues (1985) conducted a retrospective cohort mortality study of 3,199 workers at a phosphate fertilizer production facility located in Polk County, Florida. The mining and processing of phosphate ore may result in exposure to uranium and the decay products of uranium, radon daughters, and thorium. The plant commenced operations in 1953 and was still in operation at the time of publication of the report. The plant became the subject of study after reports of several cases of lung cancer. The National Institute for Occupational Safety and Health (NIOSH) selected this plant for its cohort study because it had good personnel records, had many employees, and had been in operation continuously for several decades. An industrial hygiene survey by NIOSH found that the environmental samples for uranium were below applicable exposure standards.

Employee records had limited information beyond basic demographic data but did indicate the first and last day of employment and the job assignment on those days. To obtain vital status, the authors used employees' Social Security number to link to SSA files and also to Internal Revenue Service (IRS) files and

to the files of the Florida Department of Motor Vehicles. The follow-up period (person-years at risk) lasted from the date of hire to December 31, 1979, or the date of death. To obtain the number of expected deaths, the authors grouped employees by age, sex, and race and into 5-year calendar groups according to date of hire. They calculated the expected deaths based on statistics for the entire United States. The study examined overall mortality as well as sex- and race-specific mortality rates, but did not categorize workers by job classification as a proxy for radiation exposures.

Portsmouth Uranium Enrichment Facility Workers (Brown and Bloom, 1987)

At the request of a labor union, NIOSH analyzed the causes of death in a cohort of uranium enrichment workers at the Portsmouth Uranium Enrichment Facility in Pike County, Ohio (Brown and Bloom, 1987). This plant used gaseous diffusion to enrich the uranium up to 98 percent ^{235}U. In this facility the principal compounds of concern were uranyl fluoride (formed when uranium hexafluoride comes in contact with water vapor), technetium-99 compounds, and hydrogen fluoride. Because uranyl fluoride is highly soluble in water, the principal hazard from inhalation is renal damage, in contrast to the situation in plants that create finished metal products containing uranium in the form of insoluble oxides that may deposit in the lung and regional lymph nodes. As a result, the plant performed routine periodic assays of urine to determine the level of exposure of individual workers. However, because the body excretes uranium very quickly, an individual's urine uranium level is not an accurate guide to the person's usual or cumulative exposure. Therefore, the authors of the NIOSH study used the records of urinary uranium levels to classify jobs according to the relative level of exposure.

The authors used company employment records to identify all workers at the plant from its opening in 1954 and to document their work histories. It was possible to ascertain the time that each worker spent in each job. The cohort was made up of 5,773 white male employees who worked for at least a week. Two subcohorts were formed based on the company's urinalysis data. Although the company's urinalysis data were deemed inadequate for assigning exposure levels to individuals, the authors used the data to rank the company's departments by relative degree of potential exposure. Based on the frequency of measuring urinary uranium values, the authors identified 57 departments as having some risk of exposure (100 or more urinalysis reports). The 4,876 workers in those departments made up one of the subcohorts. They then ranked the 57 departments according to the proportion of urinalyses in which the uranium concentration exceeded 50 μg/L. The second subcohort consisted of the 3,545 workers ever employed in a department that was ranked in the top 50 percentile of the 57 departments. Thus, there was an opportunity to evaluate a dose–response relationship. The authors determined vital status by searching the records of the SSA, the IRS, the U.S. Post Office, the Ohio Bureau of Motor Vehicles, and company records from 1954 to 1982. The authors determined the person-years at

risk starting with the time the person first worked in one of the at-risk departments of the plant and ending at death or in 1982. Death rates of U.S white males and Ohio state mortality rates served as comparisons. The level of exposure in this cohort was relatively low: 94 percent of reported values were below the limits of detection, 5.1 percent were between 10 and 50 μg/L, and 0.6 percent were greater than 50 μg/L. Only 50 percent worked at the plant more than 5 years, and the turnover of employees was 15–25 percent per 5 years.

Uranium Processing Workers—Linde Air Products Company (Dupree et al., 1987)

A study by Dupree and colleagues (1987) examined mortality among workers at the Linde Air Products Company Ceramics Plant in Buffalo, New York. The plant converted uranium ore to uranium tetrafluoride from 1943 to 1949. The intermediate products in the conversion process were insoluble uranium oxides. Workers also had exposure to other toxic chemicals, including sulfuric acid. Uranium ores with high content of radium-226, an external gamma radiation hazard, were present in the plant for 18 months. Workers were allowed to work for only 2 hours a day with these ores.

The study cohort (n = 995) consisted of white males who had worked at the plant for at least 30 days. The authors created an employment roster from company records and cross-checked it against IRS records (93–95 percent concordance). Worker's individual job histories were reconstructed from security records, medical examination records, and earnings records. The authors tracked the vital status of the members of the cohort through the SSA and other sources. They obtained death certificates from the Department of Energy's Death Certificate Retrieval Office and coded the cause of death. Vital status was ascertained for 94.3 percent of the workers, and death certificates were obtained for 94.6 percent of the deceased. The authors compared the death rates of the cohort with death rates for all U.S. white males and for white males in Erie and Niagara counties of New York, the counties in which the workers lived. The period during which a worker was at risk of death began at the date of hire and ended at death or on December 31, 1979, the close of the study.

The authors estimated the dose ranges for each job in the plant using information on airborne radon and uranium monitoring, surface contamination, and urine uranium levels and created a model that assumed a distribution of inhaled particle size. The jobs were grouped into three categories with estimated annual lung doses of <10 mSv, 10–100 mSv, and 100–1,000 mSv. For reference, the occupational limit for lung dose is 150 mSv/year. The authors classified jobs by external radiation dose, using film badge records. The estimated external dose during the 18 months in which radium-226 was present (<20 mSv/year) was considerably less than the allowable whole-body dose limit (50 mSv/year). Therefore, they classified workers only according to internal uranium exposure.

The cohort was relatively young, with 64 percent aged 16–35 years at the date of hire. The vast majority began work from 1943 to 1945, and 56 percent

worked less than a year. Of this cohort, 38 percent had an annual internal exposure that exceeded 100 mSv.

Fernald Feed Materials Production Center Workers (Ritz, 1999)

The Fernald Feed Materials Production Center (FFMPC) processed uranium from ore concentrate or low-grade enriched uranium into fabricated uranium metal products (Ritz, 1999). The primary radiation exposure was to ^{235}U (varying from slightly enriched to depleted uranium); thorium was present in small amounts. The author of this recent report had access to the data set from the Comprehensive Epidemiology Data Resource (CEDR) of the Department of Energy. Although the Fernald cohort was relatively small (n = 4,014), it is one of the largest cohorts that received both internal and external exposure monitoring. Furthermore, the cohort had a long follow-up period (mean, 30.9 years), which allowed for many events to occur after the 10-year lag period assumed between exposure and radiation-related solid tumors. These factors enabled the author to evaluate the data for the combined effects of internal and external exposure over a long period. Lung cancer cases in the Fernald cohort were included in an analysis (described later in this section) by Dupree and colleagues (1995).

The cohort comprised white male workers with an estimated average age at employment of 30.1 years. The cohort was employed between the opening of the plant in 1951 and when operations stopped in 1989. The author used SSA files and the National Death Index to ascertain vital status. There were 1,064 deaths, and death certificates were available for 99 percent of the deceased. Workers' salaries provided an index of socioeconomic status. Film badges were the source of external radiation exposure measurements. Individual urine bioassays and environmental sampling of uranium dust provided estimates of internal exposure, expressed as annual lung doses. The annual lung dose was part of the CEDR data set, which provided no information about the model used to calculate it from measures of urinary uranium excretion. Thus, the author acknowledged that the annual lung dose estimates were "no more than crude indicators of relative levels of exposure among Fernald workers." The author also corrected for exposure to trichloroethylene and cutting fluids, potentially carcinogenic chemicals used in uranium processing.

The author's analysis contained several unusual features. To have sufficient mortality data for a thorough dose–response analysis, the author focused on all cancers, lung cancer, and two groups of cancers thought to be radiation-associated (NRC, 1990). One group consisted of lymphopoietic and hematopoietic malignancies. The second group combined all radiation-associated solid cancers including lung, colon, esophagus, stomach, urinary tract, and brain cancers.[3] For the dose–response analysis, the author used a case-control analysis nested in a cohort design in which she matched each death with one person who

[3]Lung cancers were considered separately and also as a subset of the second group.

was still alive at the time of the index subject's death and, when possible, the index subject's age. To allow for a period of latency to correspond to the latent period before an exposure can cause a cancer, the author limited cumulative radiation dose to that experienced 0, 10, and 15 years before the index death ("lagging exposure"). The logistic regression analysis adjusted deaths for pay category (as a proxy for socioeconomic status), time since first monitored for radiation exposure, and internal and external radiation dose.

Oak Ridge Uranium Processing Workers

Uranium processing and energy research operations have been conducted at a number of facilities in Oak Ridge, Tennessee, since 1943. A total of about 45,000 persons worked at Tennessee Eastman Corporation's Oak Ridge uranium processing plant during 1943–1947. In 1947, management of the Oak Ridge facility was transferred to Union Carbide Corporation, and work shifted to the fabrication of weapons parts and research and development (Checkoway et al., 1988). At the same time, the work force underwent a nearly complete turnover. These cohorts have been particularly informative because of the large number of employees; exposure at the beginning of the nuclear industry, which therefore provides long follow-up; and the availability of data on exposure to uranium for specific departments at the plant and, later, for individuals.

Because of the importance of this resource, a series of articles provides progressively longer follow-up and more sophisticated statistical analyses. There is overlap between several of the study cohorts. Thus, the results of the Oak Ridge studies are not necessarily independent. Two of the studies (Checkoway et al., 1988; Dupree et al., 1995) had measures of internal radiation, and there is overlap in the lung cancer cases in these studies. Other studies on the Oak Ridge workers that have focused on external radiation exposures in the research laboratory or other settings include Checkoway et al., 1985; Carpenter et al., 1988; Gilbert et al., 1993; and Cardis et al., 1995.

Uranium processing workers (1943–1947) (Polednak and Frome, 1981). Of the 45,000 persons who worked at the Y-12 Oak Ridge uranium processing plant from 1943 to 1947, complete work histories based on payrolls were available for approximately 38,000 who did not remain at the plant when ownership was transferred in 1947 (Polednak and Frome, 1981). Although about half of the employees were women, this analysis was limited to men because the method used to assess mortality using computer linkage with the Social Security Administration was less complete for women. After excluding men who worked for less than 2 days or had missing information for key data, 18,869 white males were included in the cohort for analysis. The authors do not give the reason for excluding minority groups, but the number of excluded workers was apparently small.

Uranium handled at the Y-12 Oak Ridge plant was received as UO_3 from Mallinckrodt Chemical Works in Missouri until late 1945, when the plant began to receive uranium shipments in the form of UF_6 gas. Thus, the main radiation hazard

was from the inhalation of uranium compounds, rather than from external gamma radiation. The process used to enrich the ^{235}U content of uranium involved a mass spectrographic unit, with two enrichment stages that exposed workers to uranium dust. The first step enriched the ^{235}U content to about 15 percent. Records indicate that many workers had high levels of exposure to uranium dust even by standards at that time (150 $\mu g/m^3$ of ambient air). Air samples from the area involving the first enrichment stage included many readings higher than the standard; in the chemistry process area of the first stage, average levels were 250–500 $\mu g/m^3$, whereas levels averaged 25–50 $\mu g/m^3$ in the first-stage mass spectrographic area and in the second-stage area. After 1945, the high levels in the first-stage processing were reduced when UF_6 began to be used as the starting material. Although UF_6 is a gas and more soluble, it was immediately converted to less soluble oxides (UO_4 and UO_3) or to UF_4. Although data on particle size are limited, the air contained small particles (<1 μm in diameter) that can be inhaled deeply into the alveoli of the lung and transported to other organs. According to the authors, probably only a few workers used respirators. Data on urinary uranium levels were too few to provide individual exposure estimates. However, about one-third of the workers had urinary uranium levels greater than 0.05 μg/ml, which corresponds approximately to the level expected at the maximum permissible concentration of uranium dust in the air. Thus, many employees, especially those involved in the early steps of uranium processing, were heavily exposed to uranium dust.

This study addressed the hypothesis that working up to several years in areas with high average levels of uranium dust was associated with increased mortality over a period 25–30 years after employment. The authors defined subgroups of workers by the department in which an individual worked and the average levels of uranium dust in these departments. The authors used an internal comparison group of employees with minimal exposure to uranium dust (e.g., workers in buildings where uranium was not processed) and an external comparison group of all U.S. white males. As noted above, deaths (reported by 1974) were ascertained through the SSA by record linkage; the authors estimated that they identified 94 percent of the deaths by this method. The authors used total and cause-specific death rates for U.S. white males, specific for age and calendar year, to compute expected numbers of deaths for the calculation of SMRs for subgroups of employees.

Oak Ridge nuclear materials fabrication workers (1948–1974) (Checkoway et al., 1988). This analysis by Checkoway and colleagues (1988) was based on a retrospective follow-up of 6,781 white men employed in the Oak Ridge nuclear materials fabrication plant (the Y-12 plant) between 1947 and 1979. The individuals included in this study cohort were hired after 1947, and therefore the cohort does not overlap with the workers studied by Polednak and Frome (1981).

Mortality follow-up was conducted primarily using SSA records, supplemented with other sources; vital status was determined for 96 percent of workers. In this facility, exposure to uranium was primarily as airborne dust resulting

from the reduction of uranium tetrafluoride to metal, casting of the metal, wet chemistry recycling of the uranium, and extraction of the UF_4. Only men hired after 1947 and before 1974 were included, thus ensuring at least 5 years of follow-up for each person.

Compared to most earlier studies of uranium mill workers, considerable data were available on individual exposures to radiation. External radiation exposure was assessed using film badges and thermoluminescent dosimeters. Internal doses were estimated using a combination of urinalyses and in vivo counting of internally deposited uranium. Doses were low compared to those of uranium miners. For workers with known doses, 69 percent experienced less than 1 rem of external (gamma) radiation, 30.6 percent experienced 1.0–9.99 rem, and only 0.4 percent experienced greater than 10 rem. Similarly, for internal (alpha) radiation, the cumulative dose was less than 1 rem for 21 percent, 1.0–9.99 for 50 percent, and greater than 10 rem for 29 percent of the workers. Information on smoking was not available.

The authors followed 85 percent of the cohort for at least 10 years and ascertained 862 deaths. The cause of death was obtained from death certificates, and SMRs were calculated by comparison with death rates among U.S. white males and Tennessee white males. The dose–response analyses assumed both 0- and 10-year latency.

Oak Ridge uranium processing and laboratory workers (Frome et al., 1990). This study examined the mortality experience of a cohort of 28,008 white males who were employed for at least 1 month at one of three Oak Ridge facilities (two uranium enrichment facilities [Y-12 or K-25] or the research and development laboratory) from 1943 to 1947. The men were not employed at the plant at any other time after 1947. Thus, most men in the Polednak and Frome (1981) cohort were included, with the addition of other workers from the K-25 site and the research laboratory. Follow-up for mortality was from 1950 to 1980.

Occupational exposures varied in the three facilities. The laboratory produced plutonium, among other activities, and monitored workers with pocket ionization chambers and later with film badges for external radiation. There were no direct measures of internal radiation. The uranium enrichment process included exposure to enriched uranium, insoluble uranium oxides, and a variety of chemicals including fluorocarbons. Because detailed individual radiation exposure data were not available, workers were classified by the likelihood of exposure (yes, no); facility in which they worked (Y-12 uranium processing facility, X-10 research facility, and K-25 gaseous diffusion facility); and duration of employment. Other variables included socioeconomic status (unskilled, skilled, professional), decade of follow-up, and birth year (before 1910 and 1910 or later). Vital status was determined through the SSA, state records, and the Health Care Financing Administration. Death certificates were obtained to determine cause of death. The authors used three main analytic approaches: (1) standard SMR analysis, (2) evaluation of trends in SMRs over the 30-year follow-up (percentage change per year), and (3) multivariate analyses to assess the independent effects of radiation exposure and the other covariates noted above.

Four Uranium Processing Operations (Dupree et al., 1995)

Dupree and colleagues (1995) conducted a retrospectively assembled case-control study of lung cancer among employees in four uranium processing operations. Two of the groups were employed at the uranium processing plant at the Oak Ridge facility during nonoverlapping time periods and have been previously described in the studies by Polednak and Frome (1981) and by Checkoway and colleagues (1988). The third operation was the Mallinckrodt Chemical Works Uranium Division in Missouri, and the fourth was the Feed Materials Production Center in Fernald, Ohio (described in the study by Ritz, 1999). The primary exposure in common among these facilities was to alpha radiation from airborne dust containing insoluble natural uranium compounds. Enriched uranium was present at the Oak Ridge facility from 1943 to 1946, and exposure to radium and radon daughters was also possible at the Mallinckrodt facility. All operations, except the Oak Ridge facility from 1943 to 1947, also processed thorium.

The authors identified eligible cases of lung cancer (n = 787) by mortality follow-up of the employee cohorts through the end of 1982, which provided at least 30 years of follow-up for each cohort. One control was selected for each case, matching on race, gender, and birth and hire dates within 3 years. The authors required both cases and controls to have been employed for at least 6 months. By focusing on a limited number of cases and controls rather than the entire cohort, they were able to re-create each person's work history in detail to provide a quantitative individual estimate of exposure to radiation.

Using employment and occupational radiation monitoring records, data was collected, as available, on smoking history (limited to never or ever smoked because further details were not routinely available), socioeconomic status (as first pay code), and complete work history. Smoking data were obtained for 48 percent of the cases and 39 percent of the controls, with 91 percent of the cases and 75 percent of the controls identified as smokers. Using the available individual and environmental data (including air monitoring data), health physicists estimated annual radiation lung doses from deposited uranium for each person.

As discussed throughout this section there is overlap between some of the cohorts of uranium-processing workers. Table 4.4 points out the extent of overlap.

HUMAN HEALTH EFFECTS OF URANIUM

This section discusses the scientific literature on the potential associations between human health effects and exposure to uranium. The committee has reorganized the results of the population studies (described in the previous section) and other research into disease-specific subsections that discuss the results and the strengths and limitations of current knowledge. The focus of the discussion is on the organs and organ systems that are the principal sites of uranium deposition following exposure. Malignant and nonmalignant diseases of the lung are impor-

TABLE 4.4 Studies with Overlapping Cohorts

	Facility (dates of operation)					
	Oak Ridge					
Reference	TEC (1943–1947)	Y-12 (after 1947)	K-25	R&DX-10	St. Louis, Mallinckrodt (1942–1966)	Ohio, Fernald (1951–1989)
Checkoway et al., 1988		+				
Frome et al., 1990	+		+	+		
Polednak and Frome, 1981	+					
Dupree et al., 1995	+	+			+	+
Ritz, 1999*						+

NOTE: The Tennessee Eastman Company (TEC) operated the Y-12 plant in Oak Ridge as a uranium enrichment facility from 1943 to 1947. The plant (referred to here as Y-12) reopened with a largely new workforce and performed materials processing and fabrication from 1947 through at least 1982.

*Ritz performed follow-up seven years after Dupree and colleagues and identified additional lung cancer cases (raising the total from 51 to 112) in the cohort.

tant because, as noted earlier, inhaled insoluble uranium oxides lodge in the lung and the hilar lymph nodes, where they may remain for an estimated several hundred days. Cancer of the lymphoid system is another focus of attention for the same reason. Bone is one of the principal sites for the deposition of soluble forms of uranium, either after ingestion or after clearance from the lung and its lymph nodes, and uranium remains in bone for a long time. The kidney is the major site at which uranium acts as a toxic heavy metal. Effects on the nervous system are discussed, despite the dearth of evidence, because of their possible interest in relation to illnesses in Gulf War veterans. The committee discusses other organ systems only briefly due to the paucity of research in these areas.

Assessing the Evidence: Factors Influencing the Quality of Studies

The following discussion highlights several issues that the committee considered in its evaluation of the epidemiologic studies on uranium processing workers. The committee followed the principle of giving more weight to high quality studies and considered a number of factors including methodological issues (as discussed in Chapter 3), measures of exposure, comparison groups, and duration of follow-up.

Measurement of Exposure

The most convincing way to demonstrate an association between an agent and disease is to show that the incidence of the disease increases as the level of the exposure increases. This approach requires an internal unexposed comparison group, which increases the likelihood that the comparison group is otherwise similar to the exposed group. Studies of occupational exposure on which the committee relied to evaluate the effect of uranium on disease used several methods for measuring exposure. Some of the methods discussed below have serious flaws that the committee considered in the evaluation of the study. Table 4.5 categorizes the occupational studies according to the method used to measure exposure to radiation.

Direct measurement in individual workers. The preferred method for an occupational study is to measure the level of exposure directly in each worker. Radiation film badges give a measure of cumulative exposure but measure only external radiation, which is a greater concern for exposure to enriched uranium than for exposure to natural or depleted uranium. Measuring the internal dose of radiation is more difficult. The best method is mathematical modeling to infer the lung dose of uranium from measurements of uranium in the urine and/or ambient dust.

TABLE 4.5 Methods of Radiation Exposure Measurement

Direct measurement in individual workers	
	Ritz, 1999
	Dupree et al., 1995[a]
	Checkoway et al., 1988
Using work history to model cumulative exposure	
	Dupree et al., 1995[a]
	Hadjimichael et al., 1983[b]
Classifying workers by maximum exposure	
	Frome et al., 1990
	Brown and Bloom, 1987
	Dupree et al., 1987
	Polednak and Frome, 1981
No measurement of exposure	
	Stayner et al., 1985
	Waxweiler et al., 1983
	Archer et al., 1973a

[a]Dupree used direct measures of exposure at one site and modeled cumulative dose by job site at two other sites.
[b]Hadjimichael assigned each worker to the job site with the highest exposure but then calculated the cumulative exposure in that site (instead of simply making an ordinal classification of workers by work site).

However, this approach requires that the company monitored workers for radiation exposure and kept thorough records. In many of the occupational retrospective cohort studies the authors found that measurements of exposure in individual workers were not available or were unreliable. In some cases, records were incomplete, so that measurements were lacking for many workers. In other cases, the only measurements were of urinary uranium excretion. Since the body rapidly excretes uranium, urinary uranium is a measure only of exposure in the preceding several days.

Using work history to model cumulative exposure. Several authors approximated individual exposure by modeling cumulative exposure using a worker's job history within the plant and the level of exposure in each work site. They measured the level of uranium exposure in various work sites within the processing plant, using measures of urinary uranium or uranium in ambient dust. This information was used to model the cumulative lung dose per unit time in the work site. They then used plant employment records to determine the amount of time each worker spent in each job. By totaling each worker's cumulative exposure in each work site over the course of the worker's period of employment, they estimated the worker's total exposure.

The modeling approach in effect assigns to each worker the average exposure in each work site. As compared with direct measurement, this approach loses information, since workers in a given site may vary in their exposure. Any approach that blurs the distinction between individual workers' exposure levels while maintaining the distinction between workers' health outcomes will reduce the variation in the sample. This biases the study toward failing to detect an association between exposure and health outcomes.

Classifying workers by maximum exposure. This approach measures average exposure in each work site, as described in the preceding section, and combines work sites into a relatively small number of groups according to the level of exposure. In one study, the authors did not make measurements of exposure but simply used the judgment of experts to classify work sites by the extent of exposure. However, instead of estimating cumulative exposure over all work sites, this method simply assigns a worker to the work site that has the highest exposure of all the sites in which the employee worked for a minimum period of time (usually one month).

This approach is a cruder form of exposure modeling since it reduces the variation among workers' exposure levels in two ways. First, it assumes that a employee spent his or her entire period of employment in one group of work sites, when in fact the worker may have spent time in sites that varied considerably in exposure levels. Secondly, it combines sites that may vary considerably in their level of exposure. For these reasons, this approach is especially prone to false-negative results (i.e., failing to detect a dose-response relationship). However, the actual impact of the shortcomings of this approach is unknown as none of the studies estimated the probability of false-negative results.

No measurements of exposure. A study that does not classify workers according to exposure cannot use workers with low exposure as a control when estimating the health effects of high exposure. These studies must use the U.S. population, or the population of the region in which the plant is sited, as the control group. With this approach, the healthy worker effect is more likely to distort estimates of the effect of exposure on health outcomes.

Comparison Group Issues

Many of the cohort studies of occupationally-exposed workers described in this chapter compared death rates in workers to death rates in the U.S. population (or the population of the counties or states in which uranium workers lived). These studies used the standardized mortality ratio (SMR) because it is the principal means used in occupational studies to express the death rate in workers relative to individuals not exposed to the agent being studied. An increased SMR (greater than 100 with 95% confidence intervals that do not include 100) indicates the possibility of an association between an exposure and a disease. Whether an association truly exists depends on the strength of the evidence.

However, interpretation of the SMR in studies of occupational cohorts is not straightforward because of a phenomenon known as the "healthy-worker

effect" (see Chapter 3). The healthy-worker effect is a finding that workers in many industries experience a lower mortality rate than the general population, which includes people who are not employed. Presumably, this effect is the result of selecting healthy people for employment and of the need to stay healthy in order to stay employed. Thus, when comparing uranium-processing workers to the U.S. population, the SMRs for many diseases, such as cardiovascular diseases, are less than 100, indicating that the nuclear industry workers were more fit than the U.S. population. Howe and colleagues (1988) studied the healthy-worker effect and concluded that its cause was differential selection for employment and for continued employment. The healthy-worker effect appears to be smaller for cancer than for other diseases (Howe et al., 1988).

The best way to avoid the healthy-worker bias is to use an internal comparison group. However, even internal comparison groups can be subject to this bias, to the extent that less healthy workers may not stay in more physically demanding jobs or jobs (such as uranium milling) that may involve greater exposure to chemical agents. Although studies that use an internal comparison group are more valuable than those using the U.S. population or the population of a region as the comparison group, some internal comparison groups are more useful than others. It may be difficult to draw conclusions on studies that directly compare the SMRs of groups of workers who experienced different levels of radiation exposure (e.g., Polednak and Frome, 1981; Brown and Bloom, 1987; Dupree et al., 1987) because the values of confounding variables may differ between the groups.

To address these limitations, researchers use a standardized rate ratio (SRR) to assess and compare groups who experience different levels of exposure; this measure is preferable when expressing a dose–response relationship (Breslow and Day, 1987). Standardized rate ratios used in multivariate analyses adjust for inter-group differences in the value of confounding variables and provide the best means of comparing mortality rates in groups exposed to different doses of radiation. This preferred method was used in the studies by Ritz (1999), Hadjimichael et al. (1983), Dupree et al. (1995), Frome et al. (1990), and Checkoway et al. (1988). Table 4.6 outlines the different methods of internal comparisons that have been used in the studies of uranium processing workers.

Adequate Follow-Up Period

To strengthen the evidence for a true association (particularly for some health outcomes, such as most cancers), the follow-up period should allow for sufficient time after exposure for the health outcome to occur in the population of concern. There are several time-related factors. Biological latency of cancer is a factor in the delay between exposure to the putative carcinogen and the appearance of cancer. For most cancers, the lag period between exposure and diagnosis is at least 10 years, however, there are exceptions, e.g., leukemia. Eliminating study participants who died from cancer that occurred within 10 years of exposure should increase the SMR if there is a true association between expo-

sure to the agent and the cancer. Conversely, the case for an association is much weaker when the death rate relative to the U.S. population is the same whether or not the authors considered the early cancer deaths.

TABLE 4.6 Methods of Comparing Heavily Exposed Workers with Less Exposed Workers

Reference	Method of Comparing Heavily Exposed with Less Exposed Workers (internal comparison)
Ritz, 1999	Cohort study using risk set analysis and standardized rate ratios and adjusting for pay code (salaried vs. hourly), time since first monitoring, internal and external radiation dose.
Dupree et al., 1995	Case-control study (matching for race, gender, age, and hire date, facility) using conditional logistic regression to predict lung cancer mortality. The predictor variables were smoking status, pay code (a surrogate for socioeconomic status), exposure to thorium, exposure to radon.
Checkoway et al., 1988	Poisson rate regression analysis to obtain maximum likelihood ratios for causes of death classified according to cumulative radiation exposure. The internal referent group was that with the lowest cumulative dose.
Hadjimichael et al., 1983	Log-linear models to predict all-cancer mortality using year of employment, smoking, cumulative radiation exposure, previous work exposure, job type as predictor variables.
Frome et al., 1990	Poisson regression analysis to describe the joint effects, using a multiplicative main effects model, of the predictor variables (duration of employment, socioeconomic status, radiation exposure, facility, birth year, and length of follow-up) on lung cancer mortality.
Brown and Bloom, 1987	Qualitative comparison of SMRs for workers in sites with high exposure with SMRs for the entire cohort.
Dupree et al., 1987	Qualitative comparison of SMRs for workers in jobs with high exposure with SMRs for the entire cohort.
Polednak and Frome, 1981	Qualitative comparison of SMRs for workers in sites with high ambient air uranium levels with the entire group, which included workers with jobs involving no exposure.
Stayner et al., 1985	None
Waxweiler et al., 1983	None
Archer et al., 1973a	None

Furthermore, the incidence of most cancers increases with age. Two-thirds of all cases of cancer are in individuals over age 65 (Longo, 1998). Lung cancer is largely a disease of men and women past the age of 55, with the peak incidence occurring between ages 55 and 65 (Minna, 1998).

In order to accumulate enough cases to avoid false-negative conclusions, it is important for the study to have adequate statistical power, which is a function of both the follow-up time and the size of the population. Longer follow-up time will allow an examination of a range of latency periods between the exposure and the diagnosis of disease.

Table 4.7 provides information on the follow-up periods of the uranium worker studies examined in this chapter. Otherwise well-designed studies such as those of Checkoway et al. (1988) and Hadjimichael et al. (1983) suffer from a relatively short period of follow-up. The studies of Ritz (1999), Dupree et al. (1995), Frome et al. (1990), and Polednak and Frome (1981) have 25 to 30 years of follow-up.

All Cancer Deaths

Collectively, the occupational cohorts of workers exposed to uranium, both to relatively insoluble oxides and to more soluble forms, provide a substantial body of evidence to judge the effects of exposure to uranium on cancer risk. Many of these workers may have had high levels of exposure during the early years of the nuclear industry and have now been followed for more than 30 years.

There is strong evidence that the levels of exposure in these occupational settings have not increased overall cancer mortality (Table 4.8). The SMRs are all close to or less than 100, indicating that cancer mortality in uranium workers was similar to the comparison group, which was either the entire U.S. male population or the population of the region near the work site. However, cancer is very heterogeneous, and organs and organ systems vary in their cumulative exposure to inhaled or ingested uranium and probably also in their susceptibility to carcinogenesis. Thus, the committee looked carefully at those cancers that were most likely to be related to internal exposure to uranium. However, we cannot exclude the possibility that exposure to uranium increases the risk of some relatively uncommon cancers.

Lung Cancer

Lung cancer has received the greatest attention in past studies of uranium exposure, and it is the disease about which the committee can make its most extensive analysis of the relationship between uranium exposure and disease. The reason for attention to lung cancer is due to the long residence of inhaled uranium dust in lung tissue and regional lymph nodes. In addition, lung cancer is a common disease and the number of cases is often sufficient to permit analysis

TABLE 4.7 Follow-up in Studies of Exposure to Uranium

Reference	Dates of Employment of Workers Who Are Eligible for Study	Date of Close of Mortality Follow-Up	Mean Follow-Up (years)	No. of Lung Cancer Deaths
Ritz, 1999	1951–1989	1990	30.9	112
Dupree et al., 1995	[a]	1983	26[b]	787
Checkoway et al., 1988	1947–1974	1979	20.6	89
Hadjimichael et al., 1983	1956–1978	1979	N/A	18[c]
Frome et al., 1990	1943–1947	1980	>33	850
Brown and Bloom, 1987	1954–1982	1982	N/A	48
Dupree et al., 1987	1943–1949	1979	30	21
Polednak and Frome 1981	1943–1947	1974	27	324
Stayner et al., 1985	1953–1977	1977	N/A	10
Waxweiler et al., 1983	1940–1972	1977	N/A	26
Archer et al., 1973a	1950–1953	1967	14	4

[a]Dupree used four different populations: two Y12 cohorts (1943–1947 cohort and 1947– cohort), Malinckrodt (1942–1966), and Fernald (1951–1989). Dupree's population and Frome's have several hundred individuals in common (the exact number is not known to the committee).
[b]Mean follow-up applies to the cases in this case-control study.
[c]There were 14 lung cancer deaths in male workers in industrial jobs (the category that is used in Table 4.9).

of subgroups that received different doses. The results of epidemiologic studies examining lung cancer mortality (Table 4.9) are discussed below.

Uranium Mill Workers on the Colorado Plateau (Archer et al., 1973a;Waxweiler et al., 1983)

The study by Archer and colleagues (1973a) was one of the first to examine cancer mortality as a result of exposure to uranium in a setting other than the enclosed, poorly ventilated spaces in an underground mine. There were 104 deaths during the 17-year period (1950–1967) of follow-up of uranium mill workers. The number of lung cancer deaths was 4 (4.26 expected) for an SMR of 94. The small cohort, short period of follow-up (maximum of 17 years), and small number of cancer patients limit this study's power to detect an increase in lung cancer deaths. In a somewhat larger cohort with up to 37 years of follow-up, Waxweiler and colleagues (1983) found fewer lung cancer deaths than expected (SMR = 83).

Phosphate Fertilizer Production Workers (Stayner et al., 1985)

The SMR for lung cancer in this study was 113 (90% CI 61–192). There was no trend to higher SMRs for lung cancer with increasing duration of employment (a proxy for dose of radiation) or length of follow-up. A trend toward higher SMRs with increasing duration of follow-up would be consistent with a biological effect of exposure because a longer period of follow-up reduces the influence of cancers detected during the first 10 years after employment. Most cancers detected in the first 10 years after exposure are not likely to be causally related to the exposure. The single exception was a trend for higher SMRs in black male employees who had been employed more than 20 years and followed over 20 years. There were only two cases of cancer in this category.

The study had several shortcomings. For most employees, the period of potential exposure was relatively brief. Fifty-four percent of the employees worked less than 6 months at the plant. Most employees were young, with 61 percent being between 18 and 28 years at the date of hire. There was no information on cigarette smoking status. The length of follow-up was too brief and the cohort still too young to accumulate enough deaths to draw any firm conclusions about the association between exposure and lung cancer. Additionally, the authors did not have precise information about job assignments and had no information about the level of radiation exposure. Therefore, workers could not be classified by the extent of their exposure. Further, the workers may have been exposed to other potentially hazardous compounds including radon daughters.

TABLE 4.8 Mortality from All Forms of Cancer

Reference	Study Site	Study Group (*n*)	No. of Observed Deaths	No. of Expected Deaths	SMR (95% CI)
Wagoner et al., 1964	Uranium mills and mines, Colorado Plateau	611[a]	6	8	75 (4–146)[b]
Archer et al., 1973a	Uranium mills, Colorado Plateau	662	20	18.11	110 (63–157)[b]
Polednak and Frome, 1981	Y-12 uranium processing plant, Oak Ridge, TN	18,869	886	1,042	85 (79–91)[b]
Waxweiler et al., 1983	Uranium mills, Colorado Plateau	2,002	82	109.8	75 (59–93)
Hadjimichael et al., 1983	Nuclear fuel fabrication plant, Connecticut	2,613[c]	40	44.5	88 (62–120)
Stayner et al., 1985	Phosphate fertilizer production facility, Florida	3,199	22	28.99	76 (51–108)[d]
Brown and Bloom, 1987	Uranium enrichment plant, Portsmouth, OH	5,773	125	146.2	85 (71–102)
Dupree et al., 1987	Uranium processing plant, Buffalo, NY	995	74	70.1	106 (83–132)
Checkoway et al., 1988	Y-12 uranium materials fabrication plant, Oak Ridge, TN	6,781	196	193.4	101 (88–117)
Frome et al., 1990	Y-12 and K-25 uranium enrichment facilities and research laboratory, Oak Ridge, TN	28,008	2,207	2,108	105 (101–109)[b]
Ritz, 1999	Uranium processing plant, Ohio	4,014	332	303.6	109 (98–122)

[a]Study Group comprised of white millers.
[b]The confidence interval was calculated by the committee; it was not stated in the study.
[c]Study Group comprised of males in industrial jobs.
[d]90% CI.

TABLE 4.9 Lung Cancer Mortality

Reference	Study Site	Study Group (*n*)	No. of Observed Deaths	No. of Expected Deaths	SMR (95% CI)	Gradient of Risk with Increased Radiation[a]	Disease Classification
Wagoner et al., 1964	Uranium mills and mines, Colorado Plateau	611[b]	0	1.9	0	Not stated	International Lists, 6th Revision: 160–164
Archer et al., 1973a	Uranium mills, Colorado Plateau	662	4	4.26	94 (–3 to 191)	Not stated	International Lists, 6th Revision: 160–164
Polednak and Frome, 1981	Y-12 uranium processing plant, Oak Ridge, TN	18,869	324	296.47	109 (97–121)[c]	No gradient of risk	Lung cancer
Hadjimichael et al., 1983	Nuclear fuel fabricating plant, Connecticut	2,613[d]	14	14.7	95 (52–160)	No gradient of risk	ICDA-8: 160–163
Waxweiler et al., 1983	Uranium mills, Colorado Plateau	2,002	26	31.4	83 (54–121)	Not stated	ICD-7: 162–163
Stayner et al., 1985	Phosphate fertilizer production plant, Florida	3,199	10	8.85	113 (61–192)[e]	Not stated	Trachea, bronchus, lung
Brown and Bloom, 1987	Uranium enrichment plant, Portsmouth, OH	5,773	48	54.6	88 (65–117)	No gradient of risk	ICD-7: 160–164

Dupree et al., 1987	Uranium processing plant, Buffalo, NY	995	21	21.7	97 (60–148)	Not stated	ICDA-8: 162–163
Checkoway et al., 1988	Y-12 uranium materials fabrication plant, Oak Ridge, TN	6,781	89	65.4[f]	136 (109–167)	Yes, if zero latency is assumed No, if 10-year latency is assumed	ICD-8: 162–163
Frome et al., 1990	Y-12 and K-25 uranium enrichment facilities and research laboratory, Oak Ridge, TN	28,008	850	667.99	127 (120–135)[c]	Not stated	ICDA-8: 162–163
Ritz, 1999	Uranium processing plant, Ohio	4,014	112	111.03	101 (83–121)	Yes	ICDA-8: 162

NOTE: ICD = *International Classification of Diseases;* ICDA = *International Classification of Diseases, Adapted*.

[a]The committee examined study results to see if there was a gradient of increased risk with increased levels of radiation.
[b]Study Group comprised of white millers.
[c]The CI was calculated by the committee; it was not stated in the study.
[d]Study Group comprised of males in industrial jobs.
[e]90% CI.
[f]The number of expected deaths was calculated by the committee; it was not stated in the study.

Portsmouth Uranium Enrichment Workers (Brown and Bloom, 1987)

This study found an SMR for lung cancer of 88 (48 observed, 54.6 expected; 95% CI 65–117). Additionally, the mortality for the two subcohorts who had higher exposure to uranium based on job categorization (as categorized by urine uranium levels) showed similar SMRs. The SMR was lower in the heavily exposed subcohorts than in the entire cohort, which is evidence against a meaningful association. There was no pattern of increasing cancer mortality with longer employment or when assuming a 15-year latency period.

The level of exposure of this cohort was relatively low. Only 50 percent worked at the plant more than 5 years, and the turnover of employees was 15–25 percent every 5 years. Further, the study did not have the power to detect small differences in increased risk of lung cancer for several reasons: the period of follow-up was relatively short (a maximum of 28 years and only 17 years for 40 percent of the cohort); according to the urinary uranium levels, relatively few workers had high levels of exposure to uranium; and members of the cohort were relatively young at the end of the follow-up period.

Uranium Processing Workers—Linde Air Products Company (Dupree et al., 1987)

There was no increase in lung cancer deaths in this cohort (21 observed, 21.7 expected; SMR 97, 95% CI 60–148). Results for lung cancer deaths were similar when the standard of comparison was white male residents of Erie and Niagara counties. Of the employees studied, 38 percent had an annual internal exposure that exceeded 100 mSv (10 rem) per year. The cohort of 995 workers was relatively young, with 64 percent aged 16–35 years at the date of hire. The vast majority of employees began work from 1943 to 1945, and 56 percent worked less than a year. A weakness of the study is the small number of workers, which means that the comparison of heavily exposed workers to less exposed workers lacked the power to detect small differences between the two groups.

Oak Ridge Workers (Polednak and Frome, 1981)

This study of workers at the Oak Ridge uranium processing plant from 1943 to 1947 found an SMR for lung cancer of 109 (95% CI 97–121). These numbers changed only slightly after corrections for incomplete ascertainment of deaths. For the 2,051 men who worked in the areas most highly exposed to uranium dust (first stage chemistry areas), the SMR was 97 for lung cancer. Lung cancer results were similar when analyses were restricted to men who had worked in heavily exposed areas for a year or more; based on 66 observed cases, the SMR was 106. Results were also similar when unexposed workers at Oak Ridge were used as the comparison group. The only suggestion of an increased risk of lung cancer was seen when men working in the heavily exposed areas were subdivided

by age at first employment. For those who were 45 years of age or more at hire the relative risk was 151 (95% CI 101–231), but for those younger than age 25 at hire the relative risk was 29 (95% CI 1–82) compared to nonexposed workers.

Because of the large size of this study and the high exposures experienced by many of the men, the lack of evidence for an increase in cancer risk is informative. However, the authors were not able to control for potentially important risk factors such as cigarette smoking, and they did not specifically test to see if cancer rates increased with longer periods of time after exposure. Further studies have evaluated this cohort (see below).

Fernald Feed Materials Production Center Workers (Ritz, 1999)

The author did not observe an increase in lung cancer deaths in this study; there were 112 observed and 111 expected deaths (SMR 101, 95% CI 83–121). However, the author made several new observations about the dose–response relationship and the possible interrelationship of external and internal radiation exposure, as shown in Table 4.10.

The author found that an external radiation dose greater than 100 mSv increased cancer mortality for all cancers, lung cancer, and radiation-associated cancers. External radiation dose increased mortality for all cancers and for lung cancer in several analyses in which the models were adjusted for exposure to cutting oil and trichlorethylene, and focused on workers whose exposure occurred after age 40, or when radiation doses were lagged by more than 10 years. Internal dose also affected cancer mortality. Internal doses of ≥200 mSv lagged by 15 years nearly doubled respiratory tract cancer mortality compared to the referent category of <10 mSv internal exposure. There were slight increases in bladder and kidney cancer deaths with increasing internal exposures, but there was no indication of an effect of increased internal radiation on hematopoietic or lymphoid cancers or colon cancer. Finally, the author combined internal and external radiation doses and showed an increase in lung cancer mortality when the internal dose exceeded 200 mSv at the same time that external dose exceeded 50 mSv.

Several aspects of this study raise concern about its interpretation. First, relatively few workers experienced high levels of external radiation exposure, with no one receiving more than 300 mSv, only 2.6 percent of the workers receiving more than 100 mSv, and 69 percent receiving less than 10 mSv. Thus, the number of cases associated with substantial external radiation exposure was small. Only 12 cancer deaths occurred in workers with greater than 100-mSv *external* radiation exposure. Only 18 deaths from cancer occurred in those with greater than 100-mSv *internal* radiation exposure. Second, a doubling of the death rate was associated with an internal radiation dose increment of 100 mSv per year, a surprisingly strong effect from a very small change in radiation exposure. Third, with internal radiation dose, there was no increase in risk of respiratory cancer after taking account of the lag period. Fourth, the highest

TABLE 4.10 Combined Effects of External and Internal Radiation Dose on Lung Cancer Mortality

	Internal Dose					
	<100 mSv		≥100 and <200 mSv		≥200 mSv	
External Dose (mSv)	No. of Deaths	RR (95% CI)	No. of Deaths	RR (95% CI)	No. of Deaths	RR (95% CI)
<50						
0-year lag	84[a]	1.0	6	0.72 (0.31–1.67)	0	
15-year lag	90[a]	1.0	6	1.24 (0.53–2.89)	0	
50–99						
0-year lag	8	1.36 (0.65–2.83)	5	1.36 (0.54–3.40)	1	7.68 (1.06–55.7)
15-year lag	9	2.03 (1.0–4.14)	2	1.28 (0.31–5.32)	2	18.0 (4.32–74.9)
≥100						
0-year lag	3	2.28 (0.71–7.31)	3	1.42 (0.44–4.54)	2	5.87 (1.42–24.2)
15-year lag	1	1.53 (0.21–1.17)	1	2.01 (0.28–14.7)	1	17.7 (2.36–133)

NOTE: RR = relative risk.

[a]The referent comparison group for calculating the relative risk in the other groups.

SOURCE: Ritz, 1999.

SRRs, in workers with high levels of both external and internal radiation, occurred with one or two deaths per category (albeit with 95% CI that did not overlap 1). Nevertheless, the data, based on well-characterized exposure levels in this study, do suggest that after controlling for external dose, internal doses up to 200 mSv are not associated with excess risk of lung cancer.

The strengths of the Fernald cohort study include direct measures of individuals' radiation exposure, a sound method of analysis, and the use of internal controls. Analysis of the Fernald cohort suggests that high-level exposure to ionizing radiation increases the rate of lung cancer. However, a very small number of deceased workers received the relatively high doses of radiation (>200 mSv of internal exposure) that were the basis of the possible relationship between radiation dose and lung cancer death. Mortality ratios based on one or two deaths are statistically weak; therefore, the study's suggestive findings require replication in a larger cohort.

Oak Ridge Nuclear Materials Fabrication Workers (Checkoway et al., 1988)

This study of Oak Ridge workers from 1947–1979 found an SMR of 136 (95% CI 109–167) for lung cancer when compared with death rates in U.S. white men during 1947 to 1979. Alternate analyses assumed 0- and 10-year latencies between exposure and mortality. Internal dose–response analyses for lung cancer mortality were based on a small number of cases in each category, so that the confidence intervals were wide. When the authors assumed no latency, there were nonsignificant increases in lung cancer risk at higher exposures. When the authors assumed a 10-year latency, there was no dose–response relationship. The study did show a dose–response gradient for gamma radiation for workers who received ≥ 5 rem of alpha radiation.

This study does not provide evidence of increased cancer risks for exposure to low levels of radiation. The borderline significant increased risk of lung cancer occurred with an assumed short latency period between radiation exposure and diagnosis. Radiation-induced cancer typically shows a minimum latency of 10 years between exposure and increased incidence, and in this analysis there was no increased risk with 10 years' latency, which suggests that the modest elevation in lung cancer deaths is due to chance or to other factors. Although the radiation exposure data are a major strength of this study, the interpretation of findings for lung cancer risk is limited by the lack of data on smoking and other potential risk factors.

Oak Ridge Uranium Processing and Laboratory Workers (Frome et al., 1990)

This study of uranium enrichment facility and energy research laboratory workers found an SMR for lung cancer of 127, based on 850 observed deaths

and 667.99 expected deaths. The cohort had elevated rates of lung cancer deaths after 5 years of follow-up, and the trend increased throughout the follow-up period at a rate of 1.44 percent per year. This study did not have detailed exposure data on individual workers and measured individual exposure (yes/no) by whether or not the individual worked at least 30 days in a job or department that had contact with radioactive materials. In the multivariate analysis, the strongest predictor of lung cancer mortality was socioeconomic status; professional workers had a substantially lower rate of lung cancer mortality. Mortality due to lung cancer was not significantly associated with the exposure measure (whether or not there was exposure to radiation for 30 days or more), and the risk decreased with increasing duration of employment (this trend was significant for all cancers but not significant for lung cancer). However, the exposure measure was of limited validity since it reduced variation in workers' exposure by classifying them according to exposure in one site rather than their entire work history. Detecting an effect of an agent is more difficult when anything reduces variation in individuals' exposure.

The multivariate analysis suggests that exposure to radiation was not the explanation for the increase in lung cancer mortality since the coefficient for exposure to radiation was not significantly different from 1. Furthermore, the analysis suggests that other factors related to socioeconomic status (SES) may account for the association with lung cancer deaths. In particular, being a professional worker rather than an unskilled worker reduced the likelihood of dying from lung cancer, because this predictor variable had a large negative coefficient that was three times its standard deviation. There are a number of factors that could account for the differences in the socioeconomic variable including different rates of smoking or jobs with higher levels of exposure in lower SES individuals.

Four Uranium Processing Operations (Dupree et al., 1995)

The salient feature of this case-control study was a thorough dose–response analysis (Table 4.11). Overall, there was no apparent relationship between internal radiation dose, lagged for 10 years, and lung cancer mortality. The only suggestion of an increased risk was for a cumulative internal dose of 25 cGy or more; the relative risk was 2.05, but this figure had extremely wide confidence intervals (0.20–20.70) owing to the very small number of cases in this group. Notably, in the next highest category of exposure, 5 to <25 cGy, the relative risk was 0.64 (CI 0.37–1.12). Because smoking data were not available for all persons, it was not possible to adjust for smoking when examining the relation between exposure at 25 cGy or more and lung cancer risk. There was also no overall relationship between external radiation exposure and lung cancer deaths except when the data were restricted to the small number of persons hired at 45 years of age or older (n = 64 pairs). Even then, the confidence interval was very wide (95% CI 31–1,411), and there was no suggestion of any trend with increasing dose.

TABLE 4.11 Dose–Response Relationship for Lung Cancer and Radiation Exposure

Internal Radiation Lung Dose (cGy)	Relative Risk of Lung Cancer (95% CI)					
	0.05–	0.25–	0.5–	2.5–	5.0–	25+
Unadjusted (787 pairs)	1.03 (0.73–1.45)	0.57 (0.38–0.85)	0.85 (0.58–1.14)	0.82 (0.52–1.30)	0.64 (0.37–1.12)	2.05 (0.20–20.70)
Adjusted for smoking (166 pairs)	0.47 (0.19–1.18)	0.46 (0.16–1.28)	0.64 (0.12–1.02)	0.34 (0.12–1.02)	0.36 (0.09–1.38)	

SOURCE: Dupree et al., 1995.

This study population overlaps considerably with the studies by Ritz (1999), Checkoway and colleagues (1988), and Frome and colleagues (1990) (Table 4.4). The overlap with the Ritz study is relatively minor. Only 51 of Dupree's 787 cases of death from lung cancer were first employed at Fernald, the site of the Ritz study. On the other hand, the overlap with the study by Frome and colleagues is substantial. Five hundred and sixty-seven of Dupree's 787 cases were first employed at TEC. Frome's study population had 850 cases of lung cancer and derived 54 percent of its study population from TEC.

This study has several important strengths including its thorough dose–response analysis, large number of lung cancer cases, and thorough approach to estimating individual exposures, which are based on a variety of sources. The data provide strong evidence that lung cancer risk does not increase up to 25-cGy cumulative internal radiation exposure (primarily uranium dust in these operations). The data do not allow an informative examination of cumulative exposures greater than 25 cGy.

Conclusions on Lung Cancer

Lung cancer mortality has been the focus of attention in many cohort studies of workers employed in the uranium processing industry. Many of these studies were large and had a long period of follow-up. As shown in Table 4.9, lung cancer mortality was not increased among occupationally exposed persons in most of these cohorts, although several cohorts do show a small increase in lung cancer mortality. Because of the large number of cases of lung cancer, the committee closely analyzed the strengths and weaknesses of the studies and focused on the best quality studies in trying to form its conclusions about the effect of radiation exposure on lung cancer.

Several studies played little role in forming the committee's conclusions due to poor exposure measures or other methodologic issues. The studies by Stayner and colleagues (1985) and Archer and colleagues (1973a) did not measure exposure to radiation and had very short periods of follow-up. The study by Polednak and Frome (1981) had the largest cohort and is useful for placing an upper bound on the possible effect of radiation on relatively uncommon cancers such as bone cancer and lymphatic cancer. However, for purposes of forming a highly exposed comparison group, this study classified workers according to the job in which they had the highest exposure rather than measuring the cumulative radiation exposure of individual workers, either directly or by modeling it from the worker's job history. This approach tends to bias the study towards failing to see an effect of radiation. The studies by Hadjimichael and colleagues (1983) and Checkoway and colleagues (1988) used good methods but had relatively few cases, no doubt in part due to the relatively short period of follow-up.

The strongest studies used internal controls, used multivariate analysis to adjust for possible confounders, had at least 30 years of follow-up, and measured the cumulative radiation exposure of individual workers (with the exception of the study by Frome and colleagues [1990], which classified workers'

radiation exposure by the job that had the highest exposure). Thus, the committee placed the greatest weight on the studies of Ritz (1999), Dupree et al. (1995), and Frome et al. (1990).

In the large study of Oak Ridge employees by Frome and colleagues (1990), the entire group experienced a small increase in lung cancer mortality. Despite its shortcoming in measuring radiation exposure, the committee felt this study was important because of its large size and multivariate analysis. The analysis showed that radiation exposure was not associated with lung cancer mortality. It also demonstrated the relative importance of several confounders. Socioeconomic status strongly predicted lung cancer risk. Further, the large number of lung cancer cases (850) minimized the chance of a false-negative conclusion. Its study population does overlap with that of Dupree and colleagues (1995).

The study by Dupree and colleagues (1995) was important because it combined data from four separate studies and utilized quantitative estimates of individual cumulative exposures to uranium to form a dose–response analysis. The large number of cases of deaths from lung cancer (787) made it possible for Dupree and colleagues to perform a detailed dose–response analysis, while adjusting for confounders. The large number of lung cancer deaths also reduced the chance of a false-negative conclusion. The study population overlapped with the study populations in the studies by Ritz (1999) and Frome and colleagues (1990) (Table 4.4). The committee did not give these studies equal independent weight in forming its conclusions. The Dupree study found that the dose–response analysis did not suggest any increase in lung cancer risk up to 25 cGy. Above this level, there were too few cases to draw any conclusions.

The strongest suggestion of an association with lung cancer appeared in the recent report by Ritz (1999), in which large and statistically significant increases in lung cancer mortality occurred in the small group of workers with a cumulative internal dose of 200 mSv or more. The committee viewed this finding with caution because the subgroup with the elevated risk had only three cases of lung cancer and because the author could not adjust for cigarette smoking, which had been an important factor in the Dupree study. Further, workers in this study may also have been exposed to other sources of radiation (e.g., radium and thorium). Nevertheless, the data based on the well-characterized exposure levels in this study do suggest that after controlling for external dose, internal doses up to 200 mSv are not associated with excess risk of lung cancer.

Although studies of uranium miners have shown increased lung cancer mortality, the effect of uranium is difficult to interpret because the miners were simultaneously exposed to radon progeny, a known cause of lung cancer (NRC, 1999).

The lack of direct information on individual workers' exposure to cigarette smoke is an important shortcoming of these studies, since cigarette smoking is generally a predictor of lung cancer. However, the apparent lack of a clear association between increased uranium exposure and increased rates of lung cancer lessens the burden of this shortcoming. If there had been an apparent positive association, it would be important to understand the relative contribution of ura-

nium and cigarette smoking and the impact of exposure to other sources of radiation or other potential hazards.

The committee concludes that there is limited/suggestive evidence of no association between exposure to uranium and lung cancer at cumulative internal dose levels lower than 200 mSv or 25 cGy. However, there is inadequate/insufficient evidence to determine whether an association does or does not exist between exposure to uranium and lung cancer at higher levels of cumulative exposure.

Lymphatic Cancer

The lymphatic system is an important potential target for uranium radiation because inhaled insoluble uranium oxides can remain up to several years in the hilar lymph nodes of the lungs. Studying the effect of uranium exposure on lymphatic cancer is more difficult than studying lung cancer because lymphatic cancer is much less common. Thus, the small number of cases magnifies the effects of all of the methodological concerns cited in the foregoing discussion of lung cancer data. The small number of expected cases means wide confidence intervals for SMRs and far too few cases for the subgroup analyses that are necessary to establish useful dose–response relationships.

Lymphomas are neoplastic transformations of cells that reside principally in lymph nodes. In view of the localization of inhaled uranium oxides in lung lymph nodes, uranium should be associated with malignant lymphomas if it is associated with any lymphoid malignancy. Table 4.12 shows the results of the epidemiologic studies that provide information on the relationship between uranium exposure and lymphoid malignancy. Some studies provided results on specific types of lymphoid malignancies while others grouped the data.

Studies of mill workers on the Colorado Plateau found an increase in lymphoid cancer deaths. The Archer et al. (1973a) study found an SMR of 392 in a small sample of uranium millers (4 cases observed versus 1.02 expected). The four cases were in millers who had not worked in the furnace area with the highest levels of exposure to uranium and vanadium. The Waxweiler et al. (1983) study with a larger cohort of millers found 5 observed and 4.2 expected deaths (SMR = 119, 95% CI 21–217) due to lymphosarcoma, reticulosarcoma, or Hodgkin's disease. However, four of the deaths due to lymphatic cancers were of employees with less than 5 years of employment, and the increase in the SMR was not statistically significant. Further, other potential exposures including vanadium and thorium could have caused the increased cases of lymphoid malignancy. In most of the studies listed in Table 4.12, the number of deaths due to lymphatic cancer has been small and the deviations from the expected number of deaths have been consistent with random variation. The Fernald cohort (Ritz, 1999) showed an increased SMR (129), but the confidence interval (91–177) included 100. By far the largest study was the Polednak and Frome (1981) report of the Oak Ridge experience, which included the period early in the nuclear industry in which workers were exposed to

relatively high amounts of inhaled uranium. In that study there were 37 deaths compared to 61 expected (SMR = 61).

Conclusions on Lymphatic Cancer

The number of cases is too small and the confidence intervals for SMRs are too wide to draw any conclusions about the association between uranium exposure and lymphoid malignancy. In particular, it is not possible to do subgroup analyses linking different levels of uranium exposure to the death rate from lymphoid malignancy. In this instance, where the evidence is all epidemiological in nature, concluding that an association may not exist would require some evidence that the incidence of lymphoid malignancy remains the same as the level of exposure increases.

The committee concludes that there is inadequate/insufficient evidence to determine whether an association does or does not exist between exposure to uranium and lymphatic cancer.

Bone Cancer

Like the lymphatic system, bone is an important potential target for the effects of uranium because uranium is distributed to the bone, replaces calcium in bone matrix, and may remain in the bone for several years. Studying the effects of uranium exposure on bone cancer is even more difficult than studying lymphoid malignancy because bone cancer is rarer, which means wide confidence intervals for the SMRs and far too few cases to establish useful dose–response relationships. The studies of bone cancer are listed in Table 4.13. According to the BEIR IV report (NRC, 1988), if there were carcinogenic effects in humans from exposure to uranium it would most likely result in increased risk of bone sarcoma (i.e., there is biological plausibility due to the deposition of uranium in bone). However, studies to date have not found an increase in bone cancers.

Conclusions on Bone Cancer

Bone cancer is rare; thus, the number of cases in all studies is small. For this reason, there is insufficient evidence to determine whether an association exists between acute or chronic exposure to uranium and bone cancer. Nevertheless, the large size of the Oak Ridge cohort (Polednak and Frome, 1981) does provide some evidence that exposure to uranium is not associated with a large excess risk of bone cancer (e.g., a relative risk of 3.0 or greater).

The committee concludes that there is inadequate/insufficient evidence to determine whether an association does or does not exist between exposure to uranium and bone cancer.

TABLE 4.12 Lymphatic Cancer Mortality

Reference	Study Site	Study Group (*n*)	No. of Observed Deaths	No. of Expected Deaths	SMR (95% CI)	Disease Classification
Archer et al., 1973a	Uranium mills, Colorado Plateau	662	4	1.02	392 (194–590)[a]	International Lists, 6th Revision: 200–203, 205
Polednak and Frome, 1981	Y-12 uranium processing plant, Oak Ridge, TN	18,869	37	61	61 (35–86)[a]	Lymphosarcoma, reticulum cell sarcoma, Hodgkin's, other lymphatic
Hadjimichael et al., 1983	Nuclear fuel fabricating plant, Connecticut	2,613 males in industrial jobs	2	3.1	65 (7–234)	ICDA-8: 200–203
Waxweiler et al., 1983	Uranium mills, Colorado Plateau	2,002	5	4.2	119 (21–217)[a]	ICD-7: 200–201

Brown and Bloom, 1987	Uranium enrichment plant, Portsmouth, OH	5,773	12	7.7	156 (84–228)[a]	ICD-7: 200–201
Dupree et al., 1987	Uranium processing plant, Buffalo, NY	995	6	6.8	89 (32–193)	ICDA-8: 200–209
Checkoway et al., 1988	Y-12 uranium materials fabrication plant, Oak Ridge, TN	6,781	15	13.1	114 (59–169)	ICD-8: 200–203, 208[b]
Frome et al., 1990	Y-12 and K-25 uranium enrichment facilities and research laboratory, Oak Ridge, TN	28,008	40	48.23	83 (54–112)[a]	ICDA-8: 202–203, 208
Ritz, 1999	Uranium processing plant, Ohio	4,014	38	29.5	129 (91–177)	ICDA-8: 200–208

NOTE: ICD = *International Classification of Diseases*; ICDA = *International Classification of Diseases, Adapted*.

[a]The confidence interval was calculated by the committee; it was not stated in the study.
[b]The causes of death included were lymphosarcoma, reticulosarcoma, Hodgkin's disease, and other lymphatic cancers.

TABLE 4.13 Bone Cancer Mortality

Reference	Study Site	Study Group (*n*)	No. of Observed Deaths	No. of Expected Deaths	SMR (95% CI)	Disease Classification
Polednak and Frome, 1981	Y-12 uranium processing plant, Oak Ridge, TN	18,869	6	6.68	90 (13–167)[a]	Bone cancer
Hadjimichael et al., 1983	Nuclear fuel fabricating plant, Connecticut	2,613 males in industrial jobs	1	0.5	206 (3–1140)	ICDA-8: 170–171
Frome et al., 1990	Y-12 and K-25 uranium enrichment facilities and research laboratory, Oak Ridge, TN	28,008	11	10.35	106 (44–168)[a]	ICDA-8: 170
Ritz, 1999	Uranium processing plant, Ohio	4,014	0	0.99	0	ICDA-8: 170

NOTE: ICDA = *International Classification of Diseases, Adapted.*

[a]The confidence interval was calculated by the committee; it was not stated in the study.

Nonmalignant Renal Disease

Mortality Risk

Studies of mortality rates from nonmalignant renal disease in occupational cohorts exposed to uranium appear in Table 4.14. In the seven cohort studies of occupational exposure and mortality from renal disease, only one study shows an excess mortality from renal disease. In that study (Waxweiler et al., 1983), all of the cases were in short-term workers, and four of the six deaths occurred within 8 years of initial employment at the uranium mill, which implies that the apparent excess mortality is not related to the extent of exposure to uranium. In most of these studies, the authors measured death rates from all genitourinary causes instead of focusing on diseases of the kidney, the part of the genitourinary tract in which uranium accumulates. The largest study (Frome et al., 1990) had 52 deaths from chronic nephritis, compared with 52.65 expected deaths (SMR = 99).

Morbidity Studies

The potential effect of uranium exposure on kidney function in humans has been examined in studies with varying doses and with different routes of exposure. This information includes case reports and studies with small numbers of subjects.

Intravenous injections. Hodge and colleagues (1973) described six patients with normal kidney function who were injected with uranyl nitrate (6.3–109 μg/kg over a 1- to 2-day period). Transient proteinuria and catalasuria occurred 4–6 days after the injection in the two patients who received the highest dose (>42 μg/kg). The authors used these observations to estimate the uranium dosage that is associated with minimal tubular kidney damage.

Bernard (1958) administered 4–50 mg of uranium compounds intravenously to eight terminally ill patients with brain tumors. Transient proteinuria and catalasuria occurred in patients receiving uranium doses >70.9 μg/kg. There was no evidence at autopsy of acute damage to the renal tubules. Based on these observations, the author estimated that the minimal uranium dose that will produce catalasuria and albuminuria is about 0.1 mg/kg.

Oral exposure. There are a few case studies with limited information about the effects of orally administered uranium compounds. Oral ingestion of uranyl nitrate at dosages as high as 925 mg, three times per day, did not cause abnormalities on routine urinalysis (Morrow et al., 1980). A volunteer who orally ingested 1 g of uranyl nitrate (0.47 g uranium) experienced vomiting, diarrhea, and slight albuminuria (Stopps and Todd, 1982). In the only other reported oral ingestion of uranium salts with follow-up measurements, 10.8 mg of uranyl nitrate had no effects on kidney function in four patients (Stopps and Todd, 1982).

TABLE 4.14 Nonmalignant Renal Disease Mortality

Reference	Study Site	Study Group (*n*)	No. of Observed Deaths	No. of Expected Deaths	SMR (95% CI)	Disease Classification
Polednak and Frome, 1981	Y-12 uranium processing plant, Oak Ridge, TN	18,869	30	39.14	77 (45–109)[a]	Chronic nephritis
Waxweiler et al., 1983	Uranium mills, Colorado Plateau	2,002	6	3.6	167 (60–353)	ICD-7: 592–594
Stayner et al., 1985	Phosphate fertilizer production plant, Florida	3,199	2	2.27	88 (16–277)[b]	Diseases of the genitourinary system
Brown and Bloom, 1987	Uranium enrichment plant, Portsmouth, OH	5,773	3	5.6	54 (11–156)	ICD-7: 590–594, 600, 602, 604, 610-637, 650–652
Checkoway et al., 1988	Y-12 uranium materials fabrication plant, Oak Ridge, TN	6,781	8	11.1	72 (31–142)	ICD-8: 580–629
Frome et al., 1990	Y-12 and K-25 uranium enrichment facilities and research laboratory, Oak Ridge, TN	28,008	52	52.65	99 (71–126)[a]	ICDA-8: 582
Ritz, 1999	Uranium processing plant, Ohio	4,014	3	14.25	21 (4–129)	ICDA-8: 580–629

NOTE: ICD = *International Classification of Diseases;* ICDA = *International Classification of Diseases, Adapted.*

[a]The confidence interval was calculated by the committee; it was not stated in the study.
[b]90% CI.

Occupational uranium exposure. Thun and colleagues (1985) compared kidney function in uranium mill workers (39 subjects) and a control group consisting of cement plant workers (36 subjects matched for sex, race, and age). In 115 of 535 (21 percent) of urinary uranium assays, mill workers had levels >30 μg/L (<1 μg U/g kidney weight). Uranium workers excreted more $beta_2$-microglobulin and five amino acids than the control group. The amount of proteinuria was small. The clearance of $beta_2$-microglobulin, relative to that of creatinine, increased with increasing length of time that the uranium workers had spent in the yellowcake drying and packaging area, the work area with the highest exposures to soluble uranium. The age of the workers did not account for this relationship. Serum $beta_2$-microglobulin was significantly higher in the uranium workers, an effect that was not due to reduced glomerular function, since glomerular function (serum creatinine and creatinine clearance) was the same in uranium workers and controls. The aminoaciduria was due to increased excretion of dicarboxylic amino acids and methionine by the uranium workers. The data suggest a reduction in renal proximal tubular reabsorption of amino acids and low-molecular-weight proteins, which is consistent with uranium nephrotoxicity.

The U.S. Uranium Registry reevaluated the intake and deposition of uranium in three men 38 years after they had been accidentally exposed to soluble uranium compounds in an explosion in 1944 (Kathren and Moore, 1986). The initial deposition of uranium in the lungs was approximately 40–50 mg, based on incomplete urinary excretion data that were obtained shortly after the accident (Eisenbud and Quigley, 1956). Two of the workers had extensive medical and health physics examinations 38 years after the accident. There was no detectable uranium, and the workers had no physical findings or renal function abnormalities that could be attributed to uranium exposure.

Lu and Zhao (1990) reported on renal function in a male worker 64 days after a 5-minute accidental exposure to uranium tetrafluoride powder (an estimated radioactivity of 6,905 Bq/m^3). The worker showed a significant increase in urinary protein, nonprotein nitrogen, amino acid nitrogen and creatinine, and phenolsulfonphthalein. These abnormal levels were present up to 3 years later but gradually returned to normal values.

Boback (1975) describes uranium excretion and clinical urinalysis in accidental exposures to an estimated 100–200 μg/kg of soluble forms of uranium. Despite an initial urine uranium excretion of 7–14 mg per day, there was no renal injury as measured by urinary protein, sugar, pH, specific gravity, or excretion of formed elements such as red blood cells or tubular casts.

Drinking water exposure. Zamora and colleagues (1998) compared the effects of uranium on kidney function in two communities, one of which had private wells supplied by underground water with a uranium content higher than the Canadian drinking water guideline. The authors divided the subjects into two groups: a low-exposure group (n = 20), whose drinking water contained <1 μg U/L, and a high-exposure group (n = 30), whose drinking water contained uranium levels from 2 to 781 μg U/L. In the low-exposure group, time of residence

in a locale with uranium-contaminated drinking water varied from 1 to 33 years, and in the high-exposure group, it varied from 3 to 59 years. The indicators of kidney function included urinary excretion of glucose, creatinine, protein, and $beta_2$-microglobulin (BMG). The markers for cell toxicity were alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), lactate dehydrogenase (LDH), and *N*-acetyl-beta-*D*-glucosaminidase (NAG). For males and females, the urinary glucose levels differed in the high- and low-exposure groups, and the amount increased with increasing uranium intake. Increases in ALP and BMG also correlated positively with increasing uranium intake. In contrast, there was no evidence for glomerular injury, as measured by normal serum creatinine concentration and no proteinuria. The authors suggest that intakes of uranium between 0.004 μg/kg and 9 mg/kg body weight are associated with altered kidney function but that the proximal tubule, rather than the glomerulus, is the site of this effect.

Fragments of depleted uranium. Uranium concentrations in the urine of Gulf War veterans have been found at higher levels in those with retained DU shrapnel than in those without when measured at 2, 4, and 7 years after first exposure (Hooper et al., 1999; McDiarmid et al., 2000). A recent study found that levels of urinary uranium ranged from 0.01 to 30.74 μg/g creatinine[4] in veterans with retained shrapnel fragments (McDiarmid et al., 2000). The concentration of uranium in the urine of nonexposed veterans ranged from 0.01 to 0.047 μg/g creatinine. Despite much higher levels of urinary uranium in the veterans with retained fragments of DU, renal function parameters (serum creatinine, BMG, and retinol-binding and urine proteins) were the same in the two groups, strongly suggesting that years of exposure to uranium does not damage the kidneys (McDiarmid et al., 2000).

Conclusions on Nonmalignant Renal Disease

Although uranium is a heavy metal that causes transient renal dysfunction, the preponderance of evidence indicates little or no clinically important renal effects of exposure to uranium. A few studies have shown changes in renal function (Lu and Zhao, 1990; Zamora et al., 1998), but the number of cases has been quite small. Perhaps the strongest evidence is the absence of kidney damage in workers that had been exposed to high levels of soluble uranium compounds (Kathren and Moore, 1986) and in veterans exposed to DU from embedded shrapnel. Kidney function was normal in Gulf War veterans with embedded DU fragments, years after exposure, despite urinary uranium concentrations up to 30.74 μg/g creatinine (McDiarmid et al., 2000).

[4]The unit of measurement for urinary uranium is expressed as micrograms per gram creatinine.

The committee concludes that there is limited/suggestive evidence of no association between exposure to uranium and clinically significant renal dysfunction.

Nonmalignant Neurological Disease

The committee carefully examined the studies on neurological outcomes as these outcomes are of interest in the study of Gulf War-related illnesses. Uranium has been shown in several animal studies to enter the brain of animals exposed through either inhalation or implantation of fragments of depleted uranium (see toxicology section). The mortality experiences of uranium processing workers (Table 4.15) generally show no excess neurologic disease mortality risks, with the exception of one study in which workers at a nuclear fuels fabrication plant had an SMR of 346 (95% CI 126–753) for death from diseases of the nervous system (Hadjimichael et al., 1983). There were 6 deaths from diseases of the central and peripheral nervous system and only 1.7 expected deaths. However, the number of cases was small, and the 95% CI was very wide. It is important to note that mortality is not a good measure for neurologic outcomes as they may not be the cause of death noted on the death certificate.

Several case studies have examined neurological outcomes or symptoms. Moore and Kathren (1985) studied three individuals 38 years after they were exposed to high concentrations of uranium (estimates of initial lung deposition of 40–50 mg of uranium) after an industrial accident. Shortly after the accident an examination found "mental status changes believed in excess of what which would be caused by fear reaction." No other details were provided. Examination of two of these individuals 38 years after the accident revealed no clinical findings attributable to uranium exposure (Moore and Kathren, 1985; Kathren and Moore, 1986). Neurological symptoms were also absent in an examination of a male worker 6 days after he had a 5-minute accidental exposure to uranium tetrafluoride powder (an estimated radioactivity of 197 nCi/m^3; 6,905.6 Bq/m^3) (Lu and Zhao, 1990).

A case report described a 44-year-old man who developed foot cramps, leg pain, a gait disorder, and a tendency to fall backward (Goasguen et al., 1982). The symptoms progressed and he developed an extrapyramidal syndrome with ataxia, nystagmus, and peripheral neuropathy. Although the authors claimed that the etiology of his illness was related to a bar of metallic uranium that he handled frequently during the first 3 years of his illness, they presented no estimates of the level of exposure of this patient to uranium and did not make a convincing argument for its causal role in his illness.

The committee found no studies of neurological symptoms after human exposure to uranium by either the oral or the dermal route.

McDiarmid and colleagues (2000) studied a cohort of Gulf War veterans who had fragments of depleted uranium in their soft tissues. As noted in the preceding section, the veterans excreted substantial amounts of uranium, presuma-

TABLE 4.15 Nonmalignant Neurologic Disease Mortality

Reference	Study Site	Study Group (*n*)	No. of Observed Deaths	No. of Expected Deaths	SMR (95% CI)	Disease Classification
Polednak and Frome, 1981	Y-12 uranium processing plant, Oak Ridge, TN	18,869	38	49.3	77 (49–105)[a]	Diseases of the nervous system
Hadjimichael et al., 1983	Nuclear fuels fabricating plant, Connecticut	2,613 males in industrial jobs	6	1.7	346 (126–753)	ICDA-8: 340–359
Stayner et al., 1985	Phosphate fertilizer production facility, Florida	3,199	3	8.73	34 (9–89)[b]	Diseases of the nervous system
Brown and Bloom, 1987	Uranium enrichment plant, Portsmouth, OH	5,773	13	32.7	40 (21–68)	ICD-7: 330–334, 345
Frome et al., 1990	Y-12 and K-25 uranium enrichment facilities and research laboratory, Oak Ridge, TN	28,008	76	81.76	93 (71–115)[a]	ICDA-8: 320–389

NOTE: ICD = *International Classification of Diseases;* ICDA = *International Classification of Diseases, Adapted.*

[a]The confidence interval was calculated by the committee; it was not stated in the study.
[b]90% CI.

bly as a result of gradual dissolution of DU fragments. Results from a battery of computer-based neurocognitive tests suggest a statistical relationship between elevated urinary uranium levels and "problematic performance on automated tests assessing performance efficiency and accuracy" (McDiarmid et al., 2000). Traditional tests of neurocognitive function (pen-and-pencil tests) did not show any statistical differences in performance between the veteran cohort and a control group.

The committee found several methodological issues that make it difficult to draw firm conclusions from this study. The authors did not adequately define their neurocognitive testing methods or the method for deciding the expected level of performance. The procedures involved calculating two "impairment indexes" for each test subject—one for the automated and one for the traditional neurocognitive measures. They calculated the impairment indexes for the neurocognitive tests by dividing the total number of scores that were below the expected score by the total number of scores obtained from each test battery. However, the investigators did not indicate how they chose the cutoff value that defined "expected" performance, nor did they explain how they chose the decision cut points.

As acknowledged by the authors, the number of individuals with high uranium levels in urine was small, "and it appeared that a few veterans with complex histories may have contributed appreciably to the observed variance." Further studies may help explain the lack of correlation between the computer-based tests, which showed abnormalities, and the standard written tests, on which the subjects performed normally. Continued follow-up of this cohort will provide insight into any potential neurocognitive effects of depleted uranium.

In summary, the evidence regarding exposure to uranium and diseases of the nervous system is not strong enough to form a firm conclusion. In studies on Gulf War veterans, the search for evidence of neurological effects will require careful neurocognitive measurements, correlation of these with clinical dysfunction, and comparison of exposed veterans to control groups chosen to illuminate various facets of the complex exposure history of Gulf War veterans.

Conclusion on Nonmalignant Neurological Disease

> *The committee concludes that there is inadequate/insufficient evidence to determine whether an association does or does not exist between exposure to uranium and diseases of the nervous system.*

Nonmalignant Respiratory Disease

Nonmalignant respiratory effects from inhaled uranium aerosols will depend in part on where in the lung the inhaled particles come to rest. Deposition depends primarily on particle size and solubility. Particle clearance mechanisms will remove a portion of deposited particles primarily by mucociliary action, which operates in the upper respiratory tract to sweep particles up to the pharynx

where they are swallowed. Particles in the lower respiratory tract may dissolve or be ingested by macrophages. In general, more soluble uranium compounds pass into the bloodstream, while less soluble forms remain in the lung or in lymph nodes for months to years. Uranium processing workers are exposed to the more soluble forms of uranium such as uranyl fluoride and uranium tetrachloride, as well as insoluble oxides. Conversely, uranium miners are exposed principally to less soluble compounds, such as UO_3, UO_2, and U_3O_8, that remain in the lung for years before fully dissolving, being taken up by the circulation, and then excreted in the urine.

Epidemiologic studies of the respiratory effects of uranium particles are difficult to interpret because of exposures to other respiratory toxicants along with uranium. Workers in uranium processing plants may have concomitant exposure to chlorine; oxides of nitrogen; nickel; or hydrofluoric, sulfuric, or nitric acids.

Studies of mortality in occupational cohorts (Table 4.16) are of limited value for assessing nonmalignant respiratory disease for many reasons. Death certificate coding for nonmalignant respiratory disease is relatively inaccurate. Most studies used only the immediate cause of death as the outcome measure of exposure, rather than also reporting the prevalence of co-morbid, nonfatal illnesses. The studies grouped multiple diseases together which decreases the ability of a study to observe an association with one disease. Additionally, most retrospective cohort studies had no direct information about cigarette smoking, which is an important cause of several common respiratory diseases and would be an important confounding variable in interpreting the effect of uranium exposure on lung disease. Further, as noted above, many of the workers were exposed to multiple agents, many of which (e.g., silica) also cause lung disease. For nonmalignant respiratory disease it is important, but often more difficult, to also examine morbidity data.

Alpha radiation from chronically inhaled uranium dust could cause lung fibrosis that could plausibly increase the incidence and mortality from chronic respiratory disease. However, studies of workers in uranium mills and uranium miners do not indicate any substantial elevation in risk of death from nonmalignant respiratory disease. Several epidemiologic studies of uranium miners have reported excess nonmalignant respiratory disease mortality, but the authors attributed these effects to dust exposure and cigarette smoking (Archer et al., 1976b; Roscoe et al., 1995), although they were observed among nonsmoking miners (Roscoe et al., 1989). Despite potential additional exposures, the SMRs for nonmalignant lung disease were close to 100 in the miner cohorts reported by Muller and colleagues (1985), Nair and colleagues (1985), and Tomasek and colleagues (1994). A report of lung pathology in 22 cases of diffuse interstitial fibrosis among uranium miners found silicosis or anthrasilicosis in six cases. The authors attributed the fibrotic changes to radiation from radon progeny alpha particles in the remaining cases (Archer et al., 1998), but they could not exclude effects of other exposures (e.g., diesel particles, other dusts) as the cause of the fibrotic findings.

TABLE 4.16 Nonmalignant Respiratory Disease Mortality

Reference	Study Site	Study Group (*n*)	No. of Observed Deaths	No. of Expected Deaths	SMR (95% CI)	Disease Classification
Polednak and Frome, 1981	Y-12 uranium processing plant, Oak Ridge, TN	18,869	340	310.11	110 (98–121)[a]	Diseases of the respiratory system
Hadjimichael et al., 1983	Nuclear fuels fabrication plant, Connecticut	2,613[b]	6	2	303 (111–659)	ICD-8: 490–493
Stayner et al., 1985	Phosphate fertilizer production facility, Florida	3,199	5	7.93	63 (25–133)[c]	Diseases of the respiratory system
Brown and Bloom, 1987	Uranium enrichment plant, Portsmouth, OH	5,773	14	33.5	42 (23–70)	ICD-7: 470–527
Dupree et al., 1987	Uranium processing plant, Buffalo, NY	995	32	21.1	152 (104–214)	ICD-8: 46–519
Checkoway et al., 1988	Y-12 uranium materials fabrication plant, Oak Ridge, TN	6,781	37	48.9	76 (53–104)	ICD-8: 460–519
Frome et al., 1990	Y-12 and K-25 uranium enrichment facilities and research laboratory, Oak Ridge, TN	28,008	792	634.11	125 (117–133)[a]	ICDA-8: 460–519
Ritz, 1999	Uranium processing plant, Ohio	4,014	53	79.78	66 (50–87)	ICD-8: 460–519

NOTE: ICD = *International Classification of Diseases;* ICDA = *International Classification of Diseases,* Adapted.

[a]The confidence interval was calculated by the committee; it was not stated in the study.
[b]Males in industrial jobs.
[c]90% CI.

The study of Dupree and colleagues (1987) found a significant excess risk of nonmalignant respiratory disease based on 32 deaths; Frome and colleagues (1990) also observed a significantly increased risk of nonmalignant lung disease. However, the other reports, including the larger studies of Checkoway and colleagues (1988), Polednak and Frome (1981), and Ritz (1999), which showed SMRs of less than or close to 100, do not confirm those findings. Polednak and Frome (1981) showed a small but statistically significant risk (SMR = 110), but the study is important principally because it provides important evidence against a large excess risk of nonmalignant lung disease. In this cohort, exposure was probably relatively intense because it occurred during the World War II era when the control of uranium dust was less stringent. In addition, the period of follow-up was long, and the number of expected deaths was large. None of the studies were able to control for smoking, a major causal factor in chronic respiratory disease, or other occupational exposures, which limits the interpretation of the findings.

Conclusion on Nonmalignant Respiratory Disease

The committee concludes that there is inadequate/insufficient evidence to determine whether an association does or does not exist between exposure to uranium and nonmalignant respiratory disease.

Other Health Outcomes

The information on other health outcomes in humans comes primarily from case reports of workers or other individuals accidentally exposed to large doses of uranium compounds. These health outcomes have not been examined in detail in human studies.

Gastrointestinal Effects

Accidental inhalation exposure of one individual to high levels of uranium produced transient gastrointestinal distress, characterized by loss of appetite, abdominal pain, diarrhea, and pus and blood in the stool (Lu and Zhao, 1990). A case of accidental dermal exposure to uranium (Lu and Zhao, 1990) had no reported gastrointestinal effects.

Immunotoxic Effects

The human literature lacks documentation on adverse immunological or lymphoreticular effects of uranium. The detection of systemic lupus erythematosus (SLE)-typical antibodies in quartz dust-exposed uranium miners indicates a potentially higher risk for the development of systemic autoimmune disease (Conrad et al., 1996, 1998). The authors of one report detected the 16/6 idiotype,

a major cross-reactive idiotype of anti-DNA antibodies involved in the pathogenesis of experimental lupus, in every member of a cohort of uranium miners who had been exposed to quartz dust (Conrad et al., 1998). Another author reported that uranium miners were more likely to develop systemic sclerosis (scleroderma), a connective tissue disease with a wide range of clinical manifestations (Baur et al., 1996). It is important to note that exposure to silica in quartz dust may be associated with both SLE and scleroderma.

Reproductive or Developmental Effects

Only a few studies have examined the effects of uranium on human reproduction and development. A greater than predicted number of female offspring was reported in male uranium miners (Muller et al., 1967). One author reported gonadal endocrine system dysfunction, with significant reduction in testosterone levels in uranium miners (Zaire et al., 1997).

In a subgroup of Gulf War veterans with embedded DU fragments in soft tissues and muscles, semen ejaculates contained uranium (McDiarmid et al., 2000). However, the semen characteristics (volume, concentration, morphology, and functional parameters of motility) were the same in Gulf War veterans with high urinary uranium excretion as in veterans with low excretion. The study also evaluated reproductive endocrinological function in Gulf War veterans with DU fragments by measuring blood levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone, and prolactin (PL). The high (>0.10 μg/g creatinine) and low (<0.10 μg/g creatinine) uranium excretion groups had the same levels of LH, FSH, PL, and testosterone (McDiarmid et al., 2000). The authors performed an unusual secondary analysis in which they found higher urinary uranium excretion in men with prolactin levels above the median than in men with PL levels below the median. The committee felt that the unconventional method of analysis was of questionable validity; the primary conventional analysis showed no significant association and the post hoc analysis, which did show a difference, included only 14 men. In addition, the authors did not measure serum cortisol, a mediator of PL plasma levels, nor did they account for moment-to-moment daily variations in prolactin. Therefore, the correlation of PL levels with the uranium dose is a hypothesis-generating observation that requires further study before any conclusions can be reached about the effect of uranium on prolactin.

Hematologic Parameters

In the study by McDiarmid and colleagues (2000) of Gulf War veterans with retained fragments of DU, hematological parameters were the same when compared with nonexposed Gulf War veterans. The parameters were also the same in veterans with retained DU fragments with either high or low urinary uranium excretion. Retained DU fragments and the ensuing increased urinary

uranium excretion did not affect hematocrit, hemoglobin, or the number of platelets, lymphocytes, neutrophils, basophils, eosinophils, and monocytes.

Genotoxicity

Background frequencies of chromosomal aberrations and sister chromatid exchanges in peripheral blood lymphocytes collected and cultured from DU-exposed veterans were identical to those of nonexposed Gulf War veterans and similar to those noted in other control populations (McDiarmid et al., 2000).

Cardiovascular Effects

SMRs for cardiovascular disease in uranium workers have been consistently less than 100, implying no important effect of uranium on cardiovascular disease. The lower than expected mortality rates are probably due to the healthy-worker effect. In addition, no cardiovascular effects occurred after one intense accidental inhalational exposure in which neither blood pressure nor pulse rate increased in a man exposed to powdered uranium tetrafluoride for 5 minutes (Lu and Zhao, 1990). Although the authors did not measure the concentration and mean particle size of the inhaled aerosol, electrocardiograms and chest x-rays were normal shortly after the accident and over a 7.5-year follow-up period.

Hepatotoxicity

In a 3-year follow-up of an individual accidentally exposed to uranium tetrafluoride, serum hepatic enzyme levels and liver function tests were within normal limits (Lu and Zhao, 1990).

Dermal, Ocular, and Musculoskeletal Effects

Dermal, ocular, and musculoskeletal effects of uranium have not been reported in the literature.

Conclusion on Other Health Outcomes

The committee concludes that there is inadequate/insufficient evidence to determine whether an association does or does not exist between exposure to uranium and gastrointestinal disease, immune-mediated disease, effects on hematological parameters, reproductive or developmental dysfunction, genotoxic effects, cardiovascular effects, hepatic disease, dermal effects, ocular effects, or musculoskeletal effects.

CONCLUSIONS

In general, animal studies have provided invaluable information on the pharmacokinetics of uranium, as well as qualitative insight into the toxicology of uranium. As discussed in this chapter, the majority of the evidence on the human health effects of exposure to uranium is from studies of workers in uranium processing mills and other facilities. Few studies of Gulf War veterans have specifically focused on the effects of uranium. Additionally, the literature on uranium miners is largely not relevant to the study of uranium per se because the primary exposure of this population was to radon progeny, which are known lung carcinogens. Although the studies of uranium processing workers are useful for drawing conclusions, the study settings have inherent weaknesses. First, even studies that involved tens of thousands of workers are not large enough to identify small increases in the relative risk of uncommon cancers. Second, few studies had accurate information about individual exposure levels. Some authors estimated the cumulative dose by following an employee's path through various jobs whose average radiation exposure was known. Third, in these industrial settings, the populations could have been exposed to other radioisotopes (e.g., radium ore, thorium) and to a number of industrial chemicals that may confound health outcomes. Finally, no studies had reliable information about cigarette smoking, which may also confound outcomes of lung cancer. However, these cohorts of uranium processing workers are an important resource, and the committee encourages further studies that will provide progressively longer follow-up, improvements in exposure estimation, and more sophisticated statistical analyses. The committee makes recommendations in Chapter 8 related to research on depleted uranium.

The following is a summary of the chapter's conclusions:

The committee concludes that there is limited/suggestive evidence of no association between exposure to uranium and the following health outcomes:

- *lung cancer at cumulative internal dose levels lower than 200 mSv or 25 cGy, or*
- *clinically significant renal dysfunction.*

The committee concludes that there is inadequate/insufficient evidence to determine whether an association does or does not exist between exposure to uranium and the following health outcomes:

- *lung cancer at higher levels of cumulative exposure (> 200 mSv or 25 cGy),*
- *lymphatic cancer,*
- *bone cancer,*

- *nervous system disease,*
- *nonmalignant respiratory disease, or*
- *other health outcomes (gastrointestinal disease, immune-mediated disease, effects on hematological parameters, reproductive or development dysfunction, genotoxic effects, cardiovascular effects, hepatic disease, dermal effects, ocular effects, or musculoskeletal effects).*

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CONTENTS

5

Sarin

Sarin is a highly toxic nerve agent produced for chemical warfare. It was synthesized in 1937 in Germany in a quest for improved insecticides (Somani, 1992). Although its battlefield potential was soon recognized, Germany refrained during World War II from using its stockpiles. Sarin's first military use did not occur until the Iran–Iraq conflict in the 1980s (Brown and Brix, 1998).

Exposure to sarin can be fatal within minutes to hours. In vapor or liquid form, sarin can be inhaled or absorbed, respectively, across the skin, eyes, or mucous membranes (Stewart and Sullivan, 1992). Because of its extreme potency, sarin is lethal to 50 percent of exposed individuals at doses of 100 to 500 mg across the skin, or 50–100 mg/min/m^3 by inhalation (in an individual weighing about 70 kg) (Somani, 1992).

Sarin is a member of a class of chemicals known as organophosphorus esters (or organophosphates). There are about 200 distinct organophosphate insecticides marketed today in thousands of formulations (Klaassen et al., 1996). A few highly toxic members of this large class are chemical warfare agents, but most are insecticides (Table 5.1) (Lotti, 2000). The drug pyridostigmine bromide (PB) is pharmacologically similar to sarin and other organophosphates, but it is a member of a different chemical class, the carbamates (see Chapter 6). Both PB and sarin exert their effects by binding to and inactivating the enzyme acetylcholinesterase (AChE). The binding of sarin to AChE is irreversible, whereas the binding of PB is reversible.

Since AChE is responsible for the breakdown of the neurotransmitter acetylcholine (ACh), the inactivation of this enzyme results in a dramatic elevation of ACh levels at cholinergic synapses (Gunderson et al., 1992). The term "cho-

linergic synapses" refers to sites throughout the body where acetylcholine exerts its actions at the synapse, or junction, between nerve cells or between nerve cells and skeletal muscles. Widespread overstimulation of muscles and nerves induced by excessive levels of acetylcholine is primarily responsible for the acute cholinergic syndrome triggered by exposure to sarin and other organophosphate (OP) nerve agents.

ACUTE CHOLINERGIC SYNDROME

In humans, exposure to high doses of sarin produces a well-characterized acute cholinergic syndrome featuring a variety of signs and symptoms affecting the peripheral and central nervous systems (Gunderson et al., 1992) (Table 5.2). The peripheral effects are categorized as either muscarinic or nicotinic, in reference to the type of receptor stimulated by acetylcholine. The muscarinic signs and symptoms usually appear first (Lotti, 2000), although the sequence of effects may vary according to the route of sarin's absorption (Stewart and Sullivan, 1992). If the dose of sarin is sufficiently high, death results after convulsions and respiratory failure (Lotti, 2000). Medical management of the acute cholinergic syndrome includes mechanical ventilation and the administration of several medications (anticholinergics, anticonvulsants, and drugs that break the chemical bond between sarin and AChE) (Sidell and Borak, 1992).

The acute health effects of sarin are exquisitely dependent on dose. Because the actual doses to humans under battlefield or terrorist circumstances cannot be measured or are difficult to reconstruct, they can be inferred on the basis of their acute clinical effects. A high level of sarin exposure of humans (after single or multiple exposures) is presumed to have occurred when the acute cholinergic syndrome is manifest. An intermediate-level exposure is presumed to have

TABLE 5.1 Examples of Organophosphates

Nerve Agents
Sarin (GB)
Soman (GD)
Tabun (GA)
Cyclosarin (GF)
o-Ethyl-*S*-[2-(diisopropylamino)ethyl]methylphosphonothiolate (VX)
Insecticides
Parathion
Malathion
Dichlorvos
Diazinon
Chlorpyrifos

TABLE 5.2 Acute Cholinergic Syndrome

Site of Action	Signs and Symptoms
Muscarinic	
Pupils	Miosis, marked, usually maximal (pinpoint), sometimes unequal
Ciliary body	Frontal headache, eye pain on focusing, blurring of vision
Nasal mucous membranes	Rhinorrhea, hyperemia
Bronchial tree	Chest tightness, prolonged wheezing, dyspnea, chest pain, increased bronchial secretion, cough, cyanosis, pulmonary edema
Gastrointestinal	Anorexia, nausea, vomiting, abdominal cramps, epigastric and substernal tightness with heartburn and eructation, diarrhea, tenesmus, involuntary defecation
Sweat glands	Increased sweating
Salivary glands	Increased salivation
Lacrimal glands	Increased lacrimation
Heart	Bradycardia
Bladder	Frequency, involuntary micturition
Nicotinic	
Striated muscle	Easy fatigue, mild weakness, muscular twitching, fasciculations, cramps, generalized weakness or flaccid paralysis (including muscles of respiration), with dyspnea and cyanosis
Sympathetic ganglia	Pallor, transitory elevation of blood pressure followed by hypotension
Central nervous system	
	Immediate (acute) effects: generalized weakness, depression of respiratory and circulatory centers with dyspnea, cyanosis, and hypotension; convulsions, loss of consciousness, and coma
	Delayed (chronic) effects: giddiness, tension, anxiety, jitteriness, restlessness, emotional lability, excessive dreaming, insomnia, nightmares, headaches, tremor, withdrawal and depression, bursts of slow waves of elevated voltage on electrogram, drowsiness, difficulty concentrating, slowness of recall, confusion, slurred speech, ataxia

SOURCE: Gunderson et al., 1992.

occurred when the acute cholinergic effect is limited to miosis (contraction of the pupil), rhinorrhea (an extreme type of runny nose), and depressed cholinesterase levels in the blood. Finally, low-level exposure may have occurred even though there are no immediately detectable cholinergic signs and symptoms (Brown and Brix, 1998). The health effects of low levels of sarin exposure are of

most interest to Gulf War veterans because of their possible exposure from demolition of Iraqi munitions at Khamisiyah, Iraq (see discussion below).

POSSIBLE U.S. TROOP EXPOSURE

In March 1991, during the cease-fire period, troops from the U.S. 37th and 307th Engineering Battalion destroyed enemy munitions throughout the occupied areas of southern Iraq (PAC, 1996). The large storage complex at Khamisiyah, Iraq, which contained more than 100 bunkers, was destroyed. Two sites within 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). U.S. troops performing demolitions were unaware of the presence of nerve agents because their detectors, which were sensitive only to lethal or near-lethal levels 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) first confirmed the presence of a mixture of sarin and cyclosarin at Khamisiyah (Committee on Veterans' Affairs, 1998).

At the request of the Presidential Advisory Committee (PAC), the Central Intelligence Agency (CIA) and the Department of Defense (DoD) conducted exposure modeling to determine the extent of exposure of U.S. military personnel to the nerve agents. Since there was no air monitoring at the time of the Khamisiyah demolition, various models were employed to develop estimates of ground level concentrations of sarin and cyclosarin as a function of distance and direction from the detonation sites (PAC, 1996). The CIA–DoD report integrated four different components: (1) UNSCOM reporting and intelligence summaries of the amount, purity, and type of chemical warfare agents stored at Khamisiyah; (2) the 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 associated type of release (instantaneous, continuous, or fly-out); (3) a combination of dispersion models, which incorporated meteorological conditions at the time (including wind direction), to simulate the transport and diffusion of the plume in order to estimate agent concentrations downwind; and (4) unit location information to determine the position of troops in relation to the plume's path (CIA–DoD, 1997). The result of this modeling effort is a series of geographic maps of the Khamisiyah area that overlays known troop unit locations with the projected path of the sarin–cyclosarin plume. According to the model, the plume includes two levels of potential exposure, the first is "a first-noticeable-effects" level (approximately 1 mg/min/m^3), where the estimated exposure was high enough to

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

cause watery eyes, runny nose, tightness of chest, muscle twitching or other early signs of chemical warfare (CW) agent exposure; the second is a lower-exposure area where the estimated dosage was less than that needed to produce first noticeable effects (CIA–DoD, 1997). The CIA–DoD report estimated that approximately 10,000 U.S. troops had been located within a 25-km radius of Khamisiyah and thus might have been exposed over a period of hours to the lower exposure level (CIA–DoD, 1997). Uncertainties with the model led to DoD's doubling these figures to 20,000 U.S. troops with possible exposure within a 50-km radius; however, the dose levels remained unaltered.

The CIA–DoD findings were challenged in a U.S. Senate report (Committee on Veterans' Affairs, 1998). The Senate report took issue with the methodology, especially the reconstruction of the pit site, the nature of the demolition, and 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 different models than those employed 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 appears to have undergone independent peer review.

DoD is conducting a complete remodeling of the Khamisiyah demolition, which is projected to be completed by the end of 2000. This remodeling, unlike the initial effort, is expected to be peer reviewed. It incorporates improved intelligence information, improved transport and diffusion modeling, and improved knowledge of unit locations. The committee encourages DoD to complete its ongoing remodeling efforts and to publish results in the peer-reviewed literature to enable broad review and independent validation of its work.

Although exposure to sarin and cyclosarin was estimated by CIA–DoD modeling, there were no medical reports by the U.S. Army Medical Corps at the time of the release that were consistent with signs and symptoms of acute exposure to sarin (PAC, 1996). Further, a 1997 survey mailed by DoD to 20,000 troops who were within a 50-km radius of Khamisiyah found that more than 99 percent of respondents (n = 7,400) reported no acute cholinergic effects (CIA–DoD, 1997). Nevertheless, low-level exposure, as noted earlier, could have occurred without producing acute cholinergic effects.

Two other storage sites in central Iraq sustained damage from air attacks during the Gulf War, but chemical agent releases were too far removed from U.S. troops for exposure to have occurred (PAC, 1996). At one site (Muhammadiyat), munitions with 2.9 metric tons of sarin–cyclosarin and 1.5 metric tons of mustard gas were damaged. At the other site (Al Muthanna), munitions containing 16.8 metric tons of sarin–cyclosarin were damaged (PAC, 1996). Atmospheric modeling by the CIA and DoD determined that the nearest U.S. personnel—located 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 U.S. demolition of a storage depot in

Khamisiyah, Iraq. The degree of exposure of U.S. troops located within the path of a sarin–cyclosarin plume, which is being remodeled in an upcoming DoD study, is at this point presumed to be low on the basis of previous exposure modeling and in the absence of medical personnel or veterans' reporting symptoms of an acute cholinergic syndrome.

The remainder of this chapter examines the scientific literature on the adverse health effects of sarin. It begins with a discussion of the toxicology of sarin and its effects on animals. It then summarizes the modest number of published toxicology studies on cyclosarin. The chapter next proceeds to its major focus, the health effects of sarin in humans. Most, if not all, toxicological and epidemiological studies focused on the health effects of sarin, as opposed to sarin in combination with other agents.

SARIN TOXICOLOGY

Sarin (GB; *o*-isopropyl methylphosphonofluoridate) is an organophosphate ester with high potency as an anticholinesterase nerve agent. It is a clear, colorless liquid with a molecular weight of 140.11, a boiling point of 158°C, and a vapor pressure of 1.48–2.9 mm Hg at 25°C (making it highly volatile). Sarin presents a liquid and a vapor hazard. In the liquid state, sarin can rapidly penetrate skin (as well as clothing), and in the vapor state it can contact the eye directly or be inhaled into the lungs, whereupon it is rapidly absorbed (Spencer et al., 2000). Exposure of the eye to vapor, which produces pinpoint pupils (miosis) and blurring of vision, accounts for one of the earliest signs of sarin exposure (Gunderson et al., 1992; Stewart and Sullivan, 1992).

Mechanisms of Acute Toxicity

Inhibition of Acetylcholinesterase

There is widespread agreement that the principal mechanism of toxicity after sarin exposure is by inhibition of acetylcholinesterase and consequent rise in ACh, leading to overstimulation at cholinergic synapses (Somani, 1992; Lotti, 2000; Spencer et al., 2000). These effects are dose related. The degree of inhibition of AChE in the mouse brain depends directly on the administered intravenous (i.v.) dose of sarin (Tripathi and Dewey, 1989). High doses of sarin (100 µg/kg) administered subcutaneously to rats produce a 32 percent increase in ACh levels (Flynn and Wecker, 1986).

Sarin inhibits AChE by phosphorylating a serine hydroxyl on the ester portion of the active site of this enzyme.[2] The phosphorylated enzyme is hydrolyzed very slowly, with a half-life of reactivation of hours to days (Gray, 1984). The

[2]During its normal function, AChE hydrolyzes acetylcholine to produce choline, acetic acid, and the reactivated enzyme. The reactivated enzyme is available to bind to another acetylcholine molecule. AChE has one of the fastest turnover rates known.

phosphorylated enzyme then can undergo a second process, called aging, by loss of an alkyl group (dealkylation). The half-life for "aging" is about 5 hours after sarin exposure (Sidell and Borak, 1992). Only during this period prior to aging can treatment with oxime therapy (e.g., pralidoxime chloride) successfully remove sarin from the enzyme and thus block the aging process. After aging has occurred, the phosphorylated enzyme (now negatively charged) is resistant to cleavage or hydrolysis and can be considered irreversibly inhibited. Recovery of AChE function occurs only with synthesis of new enzyme. Inhibition of AChE prevents the breakdown of acetylcholine, which accumulates in central and peripheral nerve synapses, leading to the acute cholinergic syndrome.

Sarin also may exert its effects through other cholinergic mechanisms (unrelated to inhibition of AChE). A new line of research suggests that sarin (in picomolar concentrations) may interact directly with muscarinic ACh receptors (Rocha et al., 1998; Chebabo et al., 1999). Researchers uncovered this new mechanism by studying sarin's ability to reduce evoked GABA (gamma-aminobutyric acid) release from hippocampal neurons. This effect of sarin is blocked by the muscarinic receptor antagonist atropine, but not by nicotinic receptor antagonists (Rocha et al., 1998). These findings suggest that sarin may interact with presynaptic muscarinic receptors, thereby reducing action potential-dependent release of GABA in the postsynaptic neuron (Chebabo et al., 1999). It is reasonable to consider that sarin acts as a muscarinic receptor antagonist inhibiting the evoked release of GABA. Reductions in the levels of GABA, which is an inhibitory neurotransmitter, may contribute to the convulsive properties of sarin.

Noncholinergic Mechanisms

For decades, researchers observed puzzling relationships between the extent of neurobehavioral toxicity and the degree of inhibition of AChE. For example, only sarin-induced tremor has a slight correlation with AChE inhibition in rat striatum, whereas chewing, hind-limb abduction, and convulsions have no clear correlation (Hoskins et al., 1986). Some sarin-treated rats with 90 percent inhibition of AChE in the striatum of the brain had no convulsions or hind-limb abduction, while rats with less enzyme inhibition exhibited both. From these findings, researchers have concluded that noncholinergic mechanisms may also contribute to toxicity induced by sarin and other organophosphates. The difficulty has been in disentangling which effects are mediated directly by sarin and which are secondary to its inhibition of AChE.

Several studies suggest that sarin may alter the level of neurotransmitters other than ACh. In most of these studies, however, the neurotransmitter effects are seen in brain regions where there are cholinergic synapses. Significant increases in catecholamines, measured histochemically, were found in the substantia nigra pars compacta and locus coeruleus of the brain following intramus-

cular (i.m.) injection of sarin at one-third of the median lethal dose (LD_{50})[3] (Dasheiff et al., 1977). Catecholamine levels in the nucleus accumbens decreased. All changes, except for the latter, returned to normal within 10 days. It is not clear whether these changes represented the direct action of sarin on enzymes related to noncholinergic neurotransmission or were secondary to the production of excessive ACh (Somani, 1992). Alternatively, stress could activate catecholamine neurons.

Levels of the neurotransmitter serotonin 5-hydroxytryptamine (5-HT) were decreased, and its major metabolite (5-hydroxyindoleacetic acid, or 5-HIAA) increased, in rat striatum after subconvulsive doses of sarin. Since this effect was also seen after administration of the OP nerve agents soman and tabun, it most likely is not agent specific, but rather is a likely consequence of an acute increase of acetylcholine in the striatum (Fernando et al., 1984).

Neuropathological damage in the hippocampus, dorsal thalamus, and piriform cortex was found in about 70 percent of rats within 24 hours of administering a single dose of sarin (95 μg/kg, i.m., or 1 LD_{50} (Kadar et al., 1995). These animals had prolonged convulsions, whereas the other 30 percent with short convulsive episodes had minimal brain damage. The authors interpreted these results to mean that convulsions may have caused the severe hypoxic damage. The neuropathology in the most affected animals continued to increase for 3 months, involving brain regions previously unaffected. The study attributed the progressive, long-term neuropathology either to delayed neurotoxicity of sarin or to secondary retrograde degeneration. It did not directly investigate potential neurochemical mechanisms underlying the neuropathology.

Toxicokinetics

This section discusses the absorption, distribution, metabolism, and elimination of sarin. In general, these events occur very rapidly after exposure, although there is some variability depending on the route of administration and the species studied (Somani, 1992). Most of the research reported here comes from animal studies, but where possible, human toxicokinetic studies are also reported.

Absorption and Metabolism

Sarin in vapor or liquid form is absorbed rapidly to produce local and systemic effects. Local effects, such as those on the eyes (e.g., miosis) and nose, are the product of sarin vapors directly interacting with AChE at the nerve endings near body surfaces (Sidell and Borak, 1992). Systemic effects, including those within the central nervous system (CNS), occur as a result of absorption of sarin into the circulation from the skin, respiratory tract, or gastrointestinal tract (Lotti, 2000).

[3] LD_{50} is the lethal dose to half or 50 percent of the test subjects.

The fate of sarin in the blood is a major determinant of how much sarin reaches the central nervous system and other sites of systemic toxicity. In the blood, sarin first interacts with several esterases (a class of enzymes). Some of the esterases, such as paraoxonase, hydrolyze sarin to inactive metabolites (Davies et al., 1996; Lotti, 2000). Two other blood esterases—AChE and butyrylcholinesterase (BuChE)—irreversibly bind to sarin. AChE found on the surface of red blood cells (RBCs), although chemically indistinguishable from AChE in the nervous system, has unknown physiological functions (Sidell and Borak, 1992). These 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 from reaching the CNS (Spencer et al., 2000). However, esterases in the blood can be overwhelmed by high doses of sarin. The acute cholinergic syndrome occurs when RBC AChE is inhibited by 75–80 percent (Sidell and Borak, 1992).

Distribution and Elimination

The tissue distribution of sarin and its metabolites has been studied in rodents. In one study a single sublethal dose (80 μg/kg) of radiolabeled sarin was administered intravenously, after which tissues were examined at distinct points in time for 24 hours (Little et al., 1986). Within 1 minute, sarin was distributed to the brain (and thus crossed the blood–brain barrier), lungs, heart, diaphragm, kidneys, liver, and plasma, with the greatest concentrations found in the last three tissues. Thereafter, the concentrations in all tissues declined. Within 15 minutes, sarin concentrations declined by 85 percent, followed by a second, more gradual decline. Relatedly, within the first minute, about half of the labeled sarin was associated with the major sarin metabolite isopropyl methylphosphonic acid (IMPA). A nonextractable label was present in constant amounts in all tissues, except plasma, throughout the time course of the experiment.

The kidneys are the major route of elimination of sarin or its metabolites. In the above study, Little and colleagues (1986) determined that kidneys contained the highest concentrations of sarin and its metabolites, whereas much lower concentrations of metabolite were detected in the liver. This suggests a minor role for the liver in detoxification of sarin. Shih and colleagues (1994) injected rats subcutaneously with a single dose of 75 μg/kg of sarin. They then measured excretion of the hydrolyzed metabolites, the alkylmethylphosphonic acids, which include IMPA and other methylphosphonic acids. Urinary elimination was found to be quite rapid; the terminal elimination half-life of sarin metabolites in urine was 3.7 ± 0.1 hours. Nearly all of the administered dose of sarin was retrieved from the urine in metabolite form after 2 days.

Distribution, metabolism, and elimination of sarin in humans appear to resemble findings in animals. Minami and colleagues (1997) detected the sarin metabolite IMPA in urine of humans after the terrorist attack on the Tokyo subway system (see later description). They found peak levels of IMPA or methylphosphonic acid in urine 10–18 hours after exposure but did not report meta-

bolic rates. The levels of IMPA in urine correlated with the degree of clinical symptoms. They also found evidence of distribution of sarin to the human brain in 4 of the 12 people who died after exposure. Solubilized sarin-bound AChE from formalin-fixed cerebellar tissue of victims of the Tokyo attack contained a derivative of the sarin hydrolysis product methylphosphonic acid (MPA) (Matsuda et al., 1998). The estimated amounts of MPA ranged from 0.32 to 1.13 nmol/g tissue. Although no IMPA was found, it was assumed that IMPA had hydrolyzed to MPA in the formalin solution over 2 years of storage.

Biomarkers of Exposure

Biomarkers of acute sarin exposure can be detected in blood or urine. In blood, the extent of inhibition of RBC AChE is considered the best marker of acute exposure. Sarin preferentially inhibits RBC AChE more than BuChE; however, after high-level sarin exposure, complete inhibition of both esterases occurs (Sidell and Borak, 1992). Since inhibition of blood cholinesterases is a common feature of organophosphates and other anticholinesterases, this biomarker is not specific to sarin exposure. Further, its utility as a biomarker is limited to a short time after exposure, with a return to original blood esterase levels by about 1–3 months (Grob, 1963). The recovery times for blood esterases are somewhat different. BuChE is replaced after about 50 days following de novo synthesis in the liver. RBC AChE recovery is contingent upon the turnover rate of red blood cells, which is about 1 percent per day. This esterase is synthesized with the RBC (Sidell and Borak, 1992). Sensitive methods for detecting urinary metabolites as biomarkers of sarin exposure were recently developed by Japanese researchers in the aftermath of the Tokyo terrorism incident (Minami et al., 1997, 1998).

Black and colleagues (1999) recently found a sensitive biomarker that can specifically identify sarin at low concentrations in human plasma. The researchers found a novel phosphonylation site, presumably from human serum albumin, at which sarin interacts with a tyrosine residue. In contrast, the biomarkers noted above are indices of sarin exposure but do not uniquely identify sarin as opposed to other CW agents. The advantage of this potentially new method is that it can directly implicate sarin at low concentrations.

Animal Studies

This section summarizes the toxic effects of sarin in laboratory animals. Most animal studies of sarin did not examine low-level exposure, but instead focused on lethal, near-lethal, or maximum tolerated doses (MTDs).[4] These high doses produced the acute cholinergic syndrome and in many cases necessitated

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

pharmacological intervention to prevent death. Although these studies enable researchers to deduce with some certainty what organ systems will not be affected by low levels of sarin (i.e., those systems that are not affected by large doses), they are not useful in distinguishing between primary damage caused by sarin and secondary damage caused by hypoxic events following convulsions.

Acute Toxicity

In animals, sarin is acutely toxic and fatal in microgram quantities in a matter of minutes. There is some variability depending on the species and the route of administration. Table 5.3 outlines the doses and routes of administration that produce acute lethality (within 24 hours) in the animal species tested. The LD_{50} in the rat and mouse are similar, with subcutaneous (s.c.), intramuscular, and intravenous doses requiring 150–180 μg/kg. Oral administration requires nearly 10 times more sarin. The hen, guinea pig, and cat are more sensitive than rats and mice, with lethal doses ranging from 16–40 μg/kg s.c. to 561 μg/kg oral.

The immediate cause of death from sarin poisoning is respiratory arrest (Rickett et al., 1986). In baboons, sarin administered to the upper airway in vapor form (30 μg/kg) causes apnea within 5 minutes (Anzueto et al., 1990). Since the dose was twice the LD_{50}, mechanical ventilation was needed to keep the animals alive. Their apnea was correlated with the absence of activity in the phrenic nerve (which projects to the diaphragm), suggesting a central effect of sarin on respiration. Respiration recovered spontaneously within 1–2 days, al-

TABLE 5.3 Acute Lethality of Sarin Administered to Various Species

Species, Strain	Route	LD_{50} (μg/kg)	Reference
Rat	s.c.	158–165	Landauer and Romano, 1984; Singer et al., 1987; Somani, 1992
Mouse, CD-1	s.c.	160–170	Clement, 1991
Mouse	i.m.	179	Somani, 1992
Mouse	i.v.	109	Little et al., 1986; Tripathy and Dewey, 1989
Mouse, Swiss albino	inhalation	600 mg/min/m^3	Husain et al., 1993
Hen	oral	561	Bucci et al., 1993
Hen	s.c.	16.5–16.7[a]	Gordon et al., 1983
Guinea pig	s.c.	53 (divided doses)	Fonnum and Sterri, 1981; Somani, 1992
Cat	s.c.	30–35	Goldstein et al., 1987

NOTE: i.m. = intramuscular; i.v. = intravenous; s.c. = subcutaneous.

[a]Converted from 0.119 μmol/kg in Ross white or Light Sussex hens.

though AChE activity was still significantly inhibited. In the cat, an infused dose of 0.56 LD_{50} caused respiratory arrest, while neuromuscular blockade required a dose in excess of five times the LD_{50} (Rickett et al., 1986). The diaphragm was still responsive to electrical stimulation at doses that inhibited respiratory nerve activity. The cells first affected were respiratory-related neurons in the medulla, and their inhibition preceded phrenic nerve inhibition. Therefore, the cause of death after sarin exposure is rapid inhibition of respiratory centers in the medulla followed by inhibition of phrenic nerve activity, which causes respiration to cease. The diaphragm muscle is paralyzed last.

Neurotoxicity

Short- and long-term neurobehavioral toxicity. Sarin's short-term behavioral effects are dose dependent. In several studies of rodents, behavior was assessed by flavor aversion, spontaneous motor activity, and motor coordination. Following subcutaneous administration of 61–115 μg/kg, sarin led to conditioned flavor aversion at doses greater than 70 μg/kg. Motor coordination, as measured by rotarod performance, was decreased at 98 μg/kg, but not at lower doses (Landauer and Romano, 1984). This study also found an increase in spontaneous locomotion at 61 μg/kg and a decrease at higher doses (measured only within 10 minutes of sarin administration). Nieminen and colleagues (1990) studied rats given intraperitoneal doses of 12.5 and 50 μg/kg, neither of which was sufficient to produce acute toxicity. By monitoring locomotor activity up to 72 hours, they found a decrease in rodent locomotion only with the highest dose until 6 hours of administration, after which time there was no difference from controls. In separate behavioral tests, they also found the highest dose of sarin to decrease certain behaviors (e.g., grooming) at 40–50 minutes after injection (Nieminen et al., 1990).

Short-term behavioral effects also have been examined in marmosets, a nonhuman primate. Doses at 33 to 55 percent of the LD_{50} disrupted the performance of animals' food-reinforced visually guided reaching response. Performance returned to normal by 24 hours 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 organophosphate 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 animal to manipulate a joystick in order to keep a rotating platform as level as possible. After administration, doses of soman, less than 2.0 μg/kg did not induce decrements in PEP performance, while doses greater than 2.75 μg/kg did induce decrements. Decrements were measured for 5 days after soman administration but later returned to normal. These findings, although not from sarin, are reported here because vestibular dysfunction has been reported as a long-term effect in humans after sarin exposure (see next section).

Long-term changes in the electroencephalogram (EEG) of rhesus monkeys occur after a single high dose of sarin (5 μg/kg, $n = 3$) or a series of 10 small doses (1 μg/kg per week, i.m., $n = 3$) (Burchfiel et al., 1976; Burchfiel and Duffy, 1982). The high dose was sufficient to produce an acute cholinergic syndrome, whereas each small dose produced few, if any, signs of acute poisoning. Animals given the large dose were pretreated with gallamine triethiodide and artificially respired to preclude the possibility of anoxic brain damage. At 24 hours after the single large dose or after the final small dose, there were significant increases in high-frequency beta activity (13–50 Hz) in the temporal lobe compared with the monkey's own pre-exposure EEGs. The increase in beta activity persisted for 1 year after sarin administration, although it did not appear to have any behavioral or psychological significance. Control animals ($n = 6$) did not exhibit any significant changes in EEG. The second component of this study, in which the same EEG change was found in humans after accidental occupational exposure to sarin, is reported later in this chapter.

A subsequent study in marmosets ($n = 17$) examined the long-term effects of a single low dose (3.0 μg/kg) of sarin on EEG and cognitive behavior (Pearce et al., 1999). In comparison with controls, which received saline injection, the sarin-dosed group experienced a 36–67 percent inhibition of RBC AChE within 3 hours. From then until 12–15 months later, no significant changes in EEG were detected, but the increase in the beta 2 amplitude (22–40 Hz) approached significance ($p = .07$). The dose did not produce a decrement in touchscreen-mediated discrimination tasks, which are indices of cognitive functioning. Pearce and colleagues attributed the discrepancy between their EEG findings and those of Burchfiel and Duffy (1982) to methodological differences. The more recent study did not use anesthesia or restraints immediately before monitoring animals' EEG.

Delayed neurotoxicity. Exposure to some, but not all, organophosphates produces a delayed neurotoxic syndrome known as organophosphate-induced delayed neuropathy (OPIDN) (Somani, 1992; Moore, 1998; Lotti, 2000). OPIDN is a progressive neuropathy that becomes manifest approximately 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). Research conducted in the 1970s determined that OPIDN results from the chemical interaction between certain organophosphates and an enzyme known as neuropathy target esterase (NTE), whose normal function in blood and other tissues is unknown. After the organophosphate covalently binds to NTE, the complex undergoes a further reaction known as aging through dealkylation of the bonded ester or amide. NTE activity in the brain typically must be decreased by 70 percent before eventual manifestation of symptoms. That different OPs produce different degrees of inhibition of NTE explains some of their variability in triggering delayed neurotoxicity. OPIDN is associated with histopathological evidence of axonal degeneration of peripheral nerves and spinal

cord. It is also associated with slightly reduced nerve conduction velocities. The specific pathophysiological steps giving rise to delayed manifestation of symptoms are not well understood (Somani, 1992; Lotti, 2000; Spencer et al., 2000; see Chapter 6 also).

In some animal models, massive doses of sarin can cause delayed neurotoxicity, which becomes manifest by ataxia and paralysis appearing days to weeks after a single high exposure or multiple lower exposures (Somani, 1992; Lotti, 2000; Spencer et al., 2000). The doses of most OPs capable of producing these 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 pharmacological and other interventions collectively referred to as "protection."

This line of research in animals is an outgrowth of historical episodes (dating back to the 1880s) of human poisoning by organophosphates. The most dramatic episode occurred in the 1930s when 20,000–40,000 people developed a delayed neurotoxicity 10–14 days after drinking an illicit alcoholic beverage containing an organophosphate contaminant (TOCP, or tri-*o*-cresyl phosphate) (De Bleecker et al., 1992).

Table 5.4 summarizes findings from animal studies of OPIDN or other forms of delayed neurotoxicity after administration of sarin. The findings are based on abnormal behaviors exhibited by the study animal. The development of delayed neurotoxicity is dependent on the animal species (e.g., hen is the species of choice because of its sensitivity to sarin), dose, route of administration, number of doses, and protection used.

In several studies, sarin did not produce delayed neurotoxicity. The negative findings in hens were attributed by Crowell and colleagues (1989) to sarin's inability to significantly inhibit brain NTE at nonlethal doses. Sarin did produce delayed neurotoxicity in six studies. In four of them, the doses were either at the lethal level or at least 30 times higher than the lethal level (Davies et al., 1960; Davies and Holland, 1972; Willems et al., 1983), or about 30–60 times the LD_{50} (Gordon et al., 1983). Animals displayed severe signs of acute cholinergic toxicity but were protected from death by administration of atropine and other agents. From these studies, most investigators concluded that sarin was unlikely to produce delayed neurotoxicity at sublethal doses.

In two more recent studies, however, sublethal doses were administered. Husain and colleagues (1993) administered sarin by inhalation (5 mg/m^3 for 20 minutes, daily for 10 days) to Swiss albino mice ($n = 6$). In this strain, the LD_{50} of sarin was 600 $mg/min/m^3$ (Husain et al., 1993). By the fourteenth day after the beginning of the study, animals developed muscular weakness of the limbs and slight ataxia. Significant inhibition of NTE was found in the brain (59 percent), spinal cord (47 percent), and platelets (55 percent), and the spinal cord exhibited pathological evidence of focal axonal degeneration. Both biochemical and morphological changes were more severe in animals ($n = 6$) exposed to the positive OP control compound mipafox (2.5 mg/kg, s.c., daily for 10 days; Husain et al., 1993). None of the changes was detected in negative control ani-

mals (n = 8) exposed to fresh air in an exposure chamber. At no time did sarin-exposed animals show signs of cholinergic toxicity, although AChE activity was inhibited by 27 percent (blood) and 19 percent (brain). A subsequent study in white leghorn hens (*Gallus domesticus*, n = 5) given subcutaneous doses of sarin (50 μg/kg, daily for 10 days) found moderate ataxia on the fourteenth day (Husain et al., 1995). The dose is reported to be one-tenth of the LD_{50} (Husain et al., 1995). NTE activity was inhibited in brain (53 percent), spinal cord (38 percent), and platelets (54 percent). Sarin caused moderate axonal degeneration and axonal swelling, while the effects of mipafox (n = 6) were much more severe (Husain et al., 1995). Platelet acetylcholinesterase activity was inhibited by 72 percent, but no indication is provided on whether cholinergic symptoms were observed. In summary, the findings of the studies reviewed indicate evidence that sarin can cause OPIDN in some animal species, particularly at doses that produce otherwise lethal effects.

Genotoxicity

In a comprehensive study of the genotoxicity of sarin, no mutagenesis, chromosomal damage, unscheduled DNA synthesis, or sister chromatid exchange was found. In vitro doses of sarin ranging from 0.2 to 200 μg/ml and in vivo exposures in rats at 360 μg/kg did not produce toxicity in any gene toxicity assays performed (Goldman et al., 1988). Klein and colleagues (1987) measured unscheduled DNA repair and synthesis in rat hepatocytes exposed to sarin. No increase in DNA synthesis was observed, but a decrease in repair synthesis was seen after administration of two different formulations of sarin (3.0×10^{-4}–2.4×10^{-3} moles [M] sarin, with different stabilizers). This study did not control for the stabilizers, and variability between experiments casts doubt on these results.

Sub-Chronic Toxicity

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). Rats were administered sarin in two formulations (type I with tributylamine stabilizer and type II stabilized with diisopropylcarbodiimide) at three different doses: a maximum tolerated dose, MTD/2, and MTD/4 (corresponding to 300, 150, and 75 μg/kg per day, given by gavage). Both formulations produced profound inhibition of acetylcholinesterase and some deaths. No neoplastic lesions were detected after sarin (type I), 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, 2 at 300 μg/kg.). Sarin (type II) was associated with one neoplastic lesion, a lymphoma, in one male in the high-dose group (n = 12). No studies have been conducted to catalog the effects of chronic exposure to sarin.

TABLE 5.4 Delayed Neurotoxicity of Sarin

Species	Dose (μg/kg)	Route of Administration	Frequency and/or Duration	Protection	Neurobehavioral Outcomes	Reference
Chicken	25 (1 LD_{50})	i.m.	1×/day for 26–28 days	Atropine, P2S, PAD	5/8 slight ataxia	Davies and Holland, 1972
Hen	500–2,500	i.m.	1×/day for 5 days (20% of total dose given)	Atropine, P2S	9/28 ataxia at minimum dose of 1000 μg/kg[a]	Davies et al., 1960
Hen	252	s.c.	1×	Physostigmine, atropine, P2S	0/4 ataxia	Gordon et al., 1983
	504–1,962	s.c.	1×		12/12 ataxia to paralysis	
Chicken	70.2–281	Gavage	1×	Atropine	None	Bucci et al., 1993
	23–94	Gavage	1×/week for 3 weeks	Atropine	None	
Hen	50 (1/10 LD_{50})	s.c.	1×/day for 10 days	None	Moderate ataxia[b]	Husain et al., 1995
Hen	600	i.m.	1×/day for 2 days	Atropine, Physo-stigmine, P2S	0/4 DN	Willems et al., 1983
	900		1×/day for 3 days		1/3 DN	
	1,500		1×/day for 5 days		8/9 DN	
	900		1×/day for 1 day		3/4 DN	
	1,200		1×/day for 1 day		4/4 DN	

Rat	75–300	Gavage	5×/week for 13 weeks	NA	None	Bucci and Parker, 1992; Bucci et al., 1992
Mouse	5 mg/m^3	Inhalation	20 min for 10 days	None	Slight ataxia[c]	Husain et al., 1993
Cat	1,000	s.c.	1×	Physostigmine and atropine	None	Goldstein et al., 1987
	3.5	s.c.	1×/day for 10 days	None	None[d]	
	7	s.c.	1×/day for 5 days	None	None[d]	

NOTE: DN = delayed neuropathy; i.m. = intramuscular; i.p. = intraperitoneal; i.v. = intravenous; s.c. = subcutaneous; NA = not available; PAD = dodecyl iodide salt of P2S; 2-PAM = pralidoxime chloride; P2S = pralidoxime mesylate, 2-hydroxyiminomethyl-*N*-methylpyridinium methyl methanesulfonate.

[a]No hens were ataxic at 500 µg/kg. Figures not provided for doses higher than 1,000 µg/kg.
[b]Study does not report how many of five dosed animals developed moderate ataxia.
[c]Study does not report how many of six dosed animals developed slight ataxia.
[d]No behavioral signs of neurotoxicity, but sarin decreased conduction velocity of muscle spindle afferents and altered the frequency response of primary and secondary nerve endings.

Reproductive or Developmental Toxicity

Sarin appears to produce no reproductive effects in rats, rabbits, or dogs. Pregnant female rats were administered sarin (100, 240, and 380 μg/kg per day) by gavage on gestational day (gd) 6–15 and were sacrificed on gd 20. There was no evidence of developmental toxicity related to any dose or formulation of sarin, even at doses that produced maternal toxicity and 28 percent mortality in the high dose group (LaBorde et al., 1996).

Pregnant female rabbits (New Zealand White) were studied in a similar fashion, receiving sarin on gd 6-19 and sacrificed on gd 29. None of the groups had any evidence of developmental toxicity at doses that produced maternal toxicity and 25 percent mortality in the high-dose group (LaBorde et al., 1996). Male dogs exposed to sarin vapor concentration of 10 mg/min/m^3 for 6 months successfully mated and produced normal litters (Jacobson et al., 1959).

CYCLOSARIN TOXICOLOGY

Cyclosarin (cyclohexyl methylphosphonofluoridate) also belongs to the organophosphate group of nerve agents. Like other OPs, cyclosarin exerts its toxic effects by inhibition of AChE. This section reports on the limited number of toxicological studies of cyclosarin, whereas a later section reports on a study of military volunteers exposed to anticholinesterase nerve agents, including sarin and cyclosarin.

Cyclosarin produces maximal inhibition of AChE in less than 1 minute, with inhibition rate constants of 7.4 and 3.8×10^8 M^{-1} min^{-1} for AChE and BuChE, respectively (Worek et al., 1998). The aging half-life of the cyclosarin–esterase complex is 8.7 hours for AChE and 2.2 hours for BuChE (Worek et al., 1998).

The LD_{50} for cyclosarin in mice is estimated at 243 μg/kg by subcutaneous administration (Clement, 1992). In comparison, sarin's LD_{50} in this same study was somewhat lower (170 μg/kg). In protection studies, a $3LD_{50}$ dose was used, and pralidoxime chloride (2-PAM) was found to be ineffective against cyclosarin, but the antidotes toxogonin and HI-6 are effective at higher doses than were necessary to protect against sarin.[5] The author correlated the rapid recovery of HI-6-treated mice with a 67 percent reactivation of AChE 30 minutes after cyclosarin administration.

The LD_{50} for an intramuscular dose of cyclosarin in rhesus monkeys was 46.6 μg/kg (Koplovitz et al., 1992). Animals dosed with 30–75.4 μg/kg became unconscious within 2 minutes of administration. Those that survived were able to sit in their cages by 5–12 hours, and clinical signs disappeared by 12–24 hours. The primary pathological findings in most of the animals that died soon after exposure were neuronal degeneration or necrosis of the brain and spinal cord and spinal cord hemorrhage. The most affected brain regions were the

[5]HI-6 = 1-[[[4-(aminocarbonyl)pyridinio]methoxy]methyl]-2-[(hydroxyimino)methyl]-pyridinium dichloride monohydrate.

frontal and entorhinal cortex, amygdala and caudate nuclei, hippocampus, and thalamus—regions frequently affected by organophosphate poisoning. Cardiomyopathy and skeletal muscle lesions were the primary nonneural lesions.

This study also compared the efficacy of pretreatment with pyridostigmine and treatment with atropine and either 2-PAM or HI-6 given immediately after cyclosarin administration. All animals survived lethal doses of cyclosarin regardless of the oxime they received, and all were clinically normal 24 hours after dosing. Minimal nervous system lesions were observed in these animals. Cardiomyopathy and skeletal muscle lesions were apparent in about a third of protected animals.

In a subsequent study using an identical protection paradigm in rhesus monkeys, Young and Koplovitz (1995) examined biochemical and hematological parameters. They found elevated creatine kinase, lactate dehydrogenase, aspartate and alanine transaminases, and potassium ion in both oxime treatment groups 2 days after cyclosarin poisoning. The elevated biochemical markers are indications of striated muscle damage. The blood values returned to normal at 7 days. The RBC count, hemoglobin, hematocrit, and serum protein and albumin were significantly decreased at 7 days.

SUMMARY OF TOXICOLOGY

Sarin is toxic to animals in a dose-dependent manner. Animals exposed to high doses display the same acute cholinergic syndrome as displayed by humans. The main mechanism of toxicity is through inhibition of AChE. Sarin is readily and rapidly absorbed into the circulation where it is hydrolyzed or bound to blood esterases. Sarin that is not inactivated in the blood quickly distributes to the brain and other tissues where it inhibits AChE. Massive acute doses of sarin, through the inhibition of NTE, can induce delayed neurotoxicity in some, but not all, animal species. Lower doses over longer periods may also exert this effect, but more research is needed to substantiate these findings. Long-term alterations in the EEG of nonhuman primates were found after sarin administration at high doses, as well as at doses that did not produce acute signs of toxicity. The clinical significance of the EEG changes is unclear. There is no evidence of genotoxicity or reproductive or developmental toxicity. The toxicology of cyclosarin appears to be similar to that of sarin, but few studies have been reported. There are no studies of the long-term or delayed effects of toxic interactions between sarin–cyclosarin and pyridostigmine.

HUMAN STUDIES

This section reviews studies of sarin's acute and long-term health effects on humans. Four human populations have been studied following exposure to sarin: military volunteers who were exposed several decades ago to nonlethal doses of sarin and other chemical warfare agents (NRC, 1982, 1985); industrial workers

with documented acute exposure to sarin (Duffy et al., 1979); and victims of the sarin terrorist attacks in Matsumoto City in 1994 and Tokyo in 1995 (Morita et al., 1995; Okumura et al., 1996). Other studies on military volunteers have been summarized (Marrs et al., 1996) but have not been published; thus, the latter studies were not considered by the committee in reaching its conclusions.

Given the extreme dose dependence of sarin's *acute* health effects—which are literally a matter of life and death—a key question is, Do nonlethal doses of sarin have *long-term* health effects and, if so, are they too dose dependent? The possibility of low-level sarin exposure of U.S. troops during the Gulf War has generated much interest in whether sarin has *long-term* effects after a relatively short exposure at levels that are insufficient to produce an acute cholinergic syndrome.

A major limitation of most human studies of either long- or short-term health effects is the inability to document actual exposure levels. Most studies of sarin were undertaken in the aftermath of occupational accidents or terrorist attacks. In such cases, the exposure levels were inferred from clinical effects. As explained earlier, high-level exposure is inferred from the acute cholinergic syndrome (see Table 5.2) with outcomes including miosis, rhinorrhea, apnea, convulsions, and possibly death. High-level exposure requires hospitalization or emergency treatment. Intermediate-level exposure is inferred from minimal or threshold cholinergic effects such as miosis or rhinorrhea and limited decline in cholinesterase activity measured in the blood (<20 percent). Low-level exposure can be inferred from proximity to a documented exposure with no clinically detectable cholinergic signs or symptoms or detectable change in blood cholinesterase activity (Brown and Brix, 1998).[6]

As seen in the following review, there have been relatively few human studies of sarin's long-term health effects. There are more human studies of OP insecticides, whose mechanism of action is similar to sarin. Although the committee was charged with evaluating the literature on sarin, it also examined relevant studies on OP insecticides to contribute to its understanding of sarin. The committee's evaluation of the long-term effects of OP insecticides is contained in Appendix E. The studies described there do find a higher prevalence of neurological and/or psychiatric symptoms, as measured through either self-reports or standardized questionnaires, at all levels of acute OP exposure. With intermediate- or high-level acute exposures, higher symptom reporting is supported by poorer performance on standardized neuropsychological tests several years later. With low-level acute exposures (insufficient to produce cholinergic signs and symptoms), the higher symptom reporting is not consistently supported by poor performance on standardized neuropsychological tests.

[6]The U.S. federal guidelines for general population exposure to military OP nerve agents are based on values for minimum clinical cholinergic effects (i.e., the intermediate level) reduced by a hundredfold safety factor (Brown and Brix, 1998).

Studies of Military Volunteers

U.S. Military Studies

Between 1958 and 1975, the U.S. Army studied servicemen exposed voluntarily to an array of chemical warfare agents (NRC, 1982, 1985). During the program, the Army investigated only acute short-term effects. Approximately 6,720 soldiers (between the ages of 20 and 25 years) were exposed at Edgewood Arsenal, Maryland, to one or more of 254 chemicals in five classes. About 1,406 of the soldiers were exposed to 15 anticholinesterases. Of this group, 246 were tested with sarin under different conditions (e.g., i.v. or inhalation of sarin vapor), but the committee was unable to determine the actual doses to most of the soldiers by either route. However, for approximately 10 percent of this group, i.v. doses were reported to range from 3.0 to 4.0 μg/kg, alone or in combination with other agents (NRC, 1982); twenty-one soldiers were exposed to cyclosarin.[7] The servicemen were above average in physical and mental ability.

Five years after the program ended, the Department of the Army requested that the National Research Council's (NRC's) Board on Toxicology and Environmental Health Hazards examine the possible long-term health effects in servicemen tested in the research program. In a series of reports, the NRC designed and conducted a follow-up survey and examined soldiers' hospitalization and mortality. Two comparison groups of soldiers in the testing program were used as controls (i.e., those who received no test chemicals[8] and those who received chemicals other than the one under scrutiny). The NRC results and conclusions were based primarily on anticholinesterases as a class, rather than on sarin or cyclosarin.

The NRC questionnaire contained 27 outcome variables relating to health, social adjustment, and reproductive experience of the participants. Mailed survey questionnaires were returned by 64 percent of the overall population of soldiers tested. No long-term health consequences were reported by those responding to the questionnaire, including those exposed to anticholinesterases. Nonrespondents reported having had no health problems to report, when contacted later about their reasons for not returning the questionnaire. Nevertheless, the NRC cautioned that the study had low statistical power and that the exposed group was a highly selected, healthier subset than those who were unexposed. Thus, despite no major identifiable long-term effects, the NRC concluded that "the limited information available from the follow-up on these soldiers does not permit definitive conclusions regarding the nature and extent of possible long-term problems resulting from chemical exposure at Edgewood" (NRC, 1985).

The NRC also reviewed Army data tapes for hospitalizations of volunteers while still in the service (1958–1983) and reviewed Veterans Administration

[7]The committee was unable to find information regarding the dose of cyclosarin that was administered to the soldier volunteers.

[8]These were soldier volunteers in the same testing program who were used in tests of equipment or of "innocuous" substances such as caffeine or alcohol.

(VA) hospitalizations occurring after Army discharge (1963–1981). Hospitalizations of exposed volunteers were not elevated in relation to both comparison groups. Conclusions in the hospitalization study were for all anticholinesterases considered as a group. There was no evidence of increased mortality rates among participants in the entire program, as well as in the subgroups of anticholinesterases. Among soldiers (n = 149) exposed to sarin (alone or in combination with other agents) the number of deaths was lower than that expected for U.S. males, based on age-specific death rates for each calendar year of follow-up. The NRC noted that the lower death rate was expected because of the "healthy-soldier effect" (see Chapter 3). It concluded that there was no evidence of a long-term effect on mortality among servicemen exposed to chemical warfare agents.

The Institute of Medicine's Medical Follow-Up Agency is currently conducting a follow-up study on the cohort of soldiers experimentally exposed to sarin and other anticholinesterase chemical warfare agents at Edgewood to further examine possible long-term health effects attributable to that exposure.

U.K. Military Study

One of the clinical syndromes occurring after high exposure to certain OP pesticides[9] is referred to as a delayed intermediate syndrome (Senanayake and Karalliedde, 1987; Brown and Brix, 1998). It is a life-threatening paralysis of respiratory, neck, and limb muscles. It appears after recovery from the acute cholinergic syndrome, but before the expected time of onset of delayed neuropathy. The symptoms are reversible and disappear within about 2 weeks. Although the mechanisms are unknown, this condition probably results from damage to the neuromuscular junction or the muscle. There has been scant study of the intermediate syndrome after sarin exposure. In one uncontrolled study of male U.K. military volunteers (n = 8) exposed to sarin vapors at 15 mg/min/m^3, soldiers quickly displayed some signs of the acute cholinergic syndrome (e.g., miosis and depressed RBC AChE levels) (Baker and Sedgwick, 1996). Although soldiers did not experience muscular weakness, they developed a subclinical change detected by single-fiber electromyography of the forearm muscle, an increased "jitter" at 3 hours postexposure. Jitter refers to a variation in the time of onset of a second action potential within a motor unit after an initial discharge. Jitter is one indication of potential failure of transmission at the neuromuscular junction. The change in jitter in the soldiers was apparent at about 1 year, but disappeared by the second follow-up at about 2 years postexposure. The findings were interpreted by the authors as possibly a subtle indicator of the onset of the intermediate syndrome, but the intermediate syndrome itself did not become manifest.

[9]The OP insecticides were fenthion, monocrotophos, dimethoate, and methamidophos.

Accidental Exposure of Industrial Workers

One of the first studies to raise questions about possible long-term CNS effects of OPs was an uncontrolled study of industrial workers exposed in the 1950s and 1960s (Metcalf and Holmes, 1969). This case series identified long-term alterations in workers' EEG and cognition. It provided the impetus for studies in rhesus monkeys (Burchfiel et al., 1976, described earlier) and the first controlled study of long-term CNS effects in workers accidentally exposed to sarin (Duffy et al., 1979; Burchfiel and Duffy, 1982). Researchers studied a population of 77 workers with previously documented accidental exposure at a manufacturing plant and compared them to unexposed controls from the same plant (n = 38) on EEG activity. None had been exposed within a year of the study. Exposed workers had one or more exposure incidents within the previous 6 years. At the time of exposure, they had clinical signs and depressed erythrocyte cholinesterase activity (by at least 25 percent). The EEG investigation consisted of spectral analysis of tape-recorded EEGs, visual inspection of routine clinical EEGs, and visual inspection of all-night sleep EEGs. Univariate and multivariate analysis of the EEG power spectra showed significant increase in high-frequency, beta activity (15–30 Hz) in temporal, central, and occipital regions in workers exposed to sarin compared to the control group ($p < .001$). There was a discrepancy between increased amounts of slow-wave activity in the delta and theta frequency bands (0–8 Hz) seen on visual inspection of EEG and the absence of such a finding by spectral analysis for the group exposed to sarin. Analysis of all-night sleep recordings showed a significant increase in the amount of REM (rapid eye movement) sleep only in the workers exposed to sarin. The clinical significance of these 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), although methodological details of the symptom reporting were not provided. The increase in EEG beta activity in both monkeys (see earlier discussion) and humans years after acute exposure to sarin lends credence to a chronic CNS effect of sarin.

Matsumoto, Japan, Terrorist Attack

In the late evening of June 27, 1994, Japanese terrorists spread sarin vapor, using 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 acute symptoms of sarin exposure (i.e., the acute cholinergic syndrome); 58 were admitted to hospitals, 253 sought medical assistance, and 7 people died. Sarin was later detected in air and water samples by gas chromatograph-mass spectrometry (GC-MC) (Nakajima et al., 1998). Several case reports, case series, and a population-based epidemiologic study emerged from this attack on a civilian population. The population-based study, the first of its kind on sarin exposure, identified symptoms persisting up to 3

years after exposure. In all of the studies reported here, doses are inferred on the basis of clinical effects. No dose reconstruction appears to have been performed.

A case series at one of the nearby hospitals reported that 17 of 18 patients admitted soon after the attack had an average reduction of plasma cholinesterase activity of 94 percent (Suzuki et al., 1997). In a larger case series, medical records were collected for 264 people who sought treatment, and health examinations were performed on 155 residents 3 weeks postexposure (Morita et al., 1995). This case series found that severely symptomatic patients examined at 3 weeks continued to exhibit decreased activity of plasma cholinesterase and RBC AChE; reduced serum triglyceride, serum potassium, and chloride; and elevated serum creatinine kinase, leukocytes, and ketones in urine. Blood cholinesterase levels returned to normal within 3 months. Most patients recovered by 6 months. Yet two of the nine severely poisoned patients displayed epileptiform abnormalities (although details of these abnormalities and their timing were not given) (Morita et al., 1995).

In a later follow-up examination by the same research team, four of six severely poisoned patients were reported to display visual field defects, hypoxia, low-grade fever, and what were described as "epileptic electroencephalographic changes" up to 2 years postexposure (Sekijima et al., 1997). At 7 months postexposure, one patient also developed sensory polyneuropathy and reduced sensory nerve conduction velocity. The minimal clinical information reported on this single case is not consistent with classic OPIDN, which manifests primarily as a motor deficit, or a mixed motor–sensory deficit, but never as an isolated sensory deficit (Lotti, 2000). With the exception of this poorly documented case of delayed sensory neuropathy, there appear to be no other cases of delayed neurotoxicity resembling OPIDN among the numerous cases of documented accidental or experimental exposure to sarin. Nevertheless, on the basis of animal studies (see earlier), researchers assert that OPIDN is possible in individuals who are rescued from otherwise lethal doses of sarin or in those exposed to lower levels for prolonged periods (Brown and Brix, 1998; Spencer et al., 2000).

The Matsumoto incident also triggered the first population-based study of the long-term effects of a single exposure to sarin. Nakajima and colleagues (1998, 1999) surveyed all residents (n = 2,052) living within a defined geographic area surrounding the sarin release site (1,050 meters from north to south; 850 meters from east to west).[10] They mailed questionnaires at various times until 3 years after the incident. At the outset of the study (3 weeks postexposure), about 27 percent of the cohort (n = 471) was classified as "victims" based on their reports of either receiving a diagnosis or reporting symptoms of acute cholinergic syndrome. They were compared with so-called "nonvictim" controls (n = 669) who lived in the same geographic area as the victims but did not report having acute cholinergic symptoms or diagnosis.

[10]It was estimated, from police reports, that 12 liters of sarin may have been released (Nakajima et al., 1998); however, the exact amount of sarin and its purity are unknown.

At 1 year, 54 of 318 victims (17 percent) still reported being symptomatic. More than 80 percent of victims lived closest to the site of sarin release. There were no age or gender differences between those whose acute symptoms either persisted or resolved. The most common symptoms were asthenopia[11] (38/54), fatigue (35/54), blurred vision (30/54), shoulder stiffness (19/54), and asthenia[12] (18/54). At 3 years, 27.5 percent of 167 victims reported being symptomatic, compared with 5.4 percent of controls. The odds ratios were highest for fatigue, headache, and visual disturbances (asthenopia, blurred vision, and narrowing of visual field) (Table 5.5). The limitations of the study were low response rate at 3 years (41.8 percent) and possible recall bias (Nakajima et al., 1999). It must also be pointed out that the controls were not necessarily unexposed; they likely were a mixed population of unexposed and low-level exposed individuals.

The Matsumoto experience shows that direct exposure to sarin, particularly at intermediate to high levels, is associated with the acute cholinergic syndrome. In the majority of sarin victims in Matsumoto, clinical signs and symptoms of acute sarin poisoning disappeared within a matter of days or weeks if victims survived the acute effects of respiratory failure and convulsions. Follow-up population-based studies of sarin victims in Matsumoto show that significant chronic symptoms from sarin exposure persist and include visual disturbance (asthenopia, blurred vision), fatigue or asthenia, and headache. These chronic symptoms appear to be dose dependent, given the geographic exposure data and documented clinical and laboratory findings. These follow-up studies, however, lack a well-defined control population.

Tokyo, Japan, Terrorist Attack

On the morning of March 20, 1995, terrorists simultaneously released diluted sarin vapor into three convergent lines of the Tokyo subway system (Yokoyama et al., 1998c). About 5,000 people sought medical evaluation, 1,000 of whom were symptomatic and 12 of whom died (Woodall, 1997). The hospital in closest proximity to the attacks, St. Luke's International Hospital, treated the largest group of patients (n = 641) (Okumura et al., 1996; Ohbu et al., 1997). Medical staff assessed most of these patients (83 percent) as having an intermediate level of exposure based on miosis (the most common symptom), blurred vision, and headache. Seventeen percent of the patients were presumed to have had high-level exposure. This patient group, which was admitted to the hospital, had more severe cholinergic signs and symptoms including marked miosis, weakness, difficulty breathing, fasciculations, convulsions, and >20 percent depression of cholinesterase activity in the blood. Most of these patients were given standard treatment for acute sarin intoxication (atropine, pralidoxime chloride, and diazepam). Five patients were critically ill with cardiac arrest, res-

[11]Weakness or fatigue of the visual organs, accompanied by pain in the eyes.
[12]General weakness, or loss of strength or energy.

TABLE 5.5 Relationship Between Sarin Exposure and Symptoms 3 Years After the Matsumoto Incident

Symptoms	Victims (n = 167),[a] n (%)	Controls (n = 669),[b] n (%)	Odds Ratio (95% CI)
Current symptoms			
No	121 (72.5)	633 (94.6)	6.68 (4.15–10.78)
Yes	46 (27.5)[c]	36 (5.4)	
Fatigue	25 (15.0)[c]	22 (3.3)	5.18 (2.84–9.44)
Asthenia	14 (8.4)[c]	11 (1.6)	5.47 (2.44–12.29)
Shoulder stiffness	15 (9.0)[d]	25 (3.7)	2.54 (1.31–4.94)
Bad dreams	5 (3.0)	7 (1.0)	2.92 (0.92–9.32)
Insomnia	9 (5.4)[e]	15 (2.2)	2.48 (1.07-5.78)
Blurred vision	18 (10.8)[c]	13 (1.9)	6.10 (2.92–12.72)
Narrowing of visual field	6 (3.6)[e]	7 (1.0)	3.52 (1.17–10.63)
Asthenopia	40 (24.0)[c]	21 (3.1)	9.72 (5.54–17.04)
Difficulty in smoking	0 (0)	3 (0.4)	—
Husky voice	2 (1.2)	7 (1.0)	1.15 (0.24–5.57)
Slight fever	4 (2.4)[e]	2 (0.3)	8.18 (1.49–45.07)
Palpitation	5 (3.0)[e]	5 (0.7)	4.10 (1.17–14.33)
Headache	14 (8.4)[d]	7 (1.0)	8.65 (3.43–21.81)

NOTE: Values given in absolute number of patients reporting symptoms (percentages). CI = confidence interval.

[a]Victims are those who lived in the geographic area of the incident and had one or more symptoms immediately after.
[b]Controls lived in the geographic area of the incident but did not have one or more symptoms immediately after.
[c]Significant differences noted between victims and controls at: $p < .001$.
[d]Significant differences noted between victims and controls at: $p < .01$.
[e]Significant differences noted between victims and controls at: $p < .05$.

SOURCE: Adapted from Nakajima et al., 1999.

piratory arrest, or convulsions, two of whom died. Patients in the highly exposed group improved by the time of discharge except for symptoms related to sarin's effects on the eyes—ocular pain, blurred vision, and visual darkness (Okumura et al., 1996; Ohbu et al., 1997). All but five patients were discharged from the hospital by the fifth day.

More than 20 percent of the hospital staff who treated victims developed acute cholinergic symptoms from secondary exposure (Nozaki et al., 1995; Ohbu et al., 1997). Although hospital staff quickly suspected sarin intoxication in patients, they did not take appropriate precautionary measures because they were first erroneously notified by the fire department that acetonitrile was the agent. Only hours later were they notified that sarin had been implicated by GC-MS (Okumura et al., 1998a,b). An organophosphorus anticholinesterase pre-

sumed to be sarin was later confirmed in serum samples from the victims (Polhuijs et al., 1997).

Questionnaires were distributed at 1, 3, and 6 months after the incident to 610 patients seen at St. Luke's International Hospital. Almost 60 percent of 475 respondents (290 patients) still reported symptoms related to the exposure, such as fear of subways, sleep disturbance, flashbacks, nightmares, and mood changes—symptoms that the authors interpreted as indicative of posttraumatic stress disorder (PTSD; Ohbu et al., 1997).

Six to eight months later, 18 symptom-free survivors with previous intermediate- and high-level exposure to sarin were tested for persistent CNS effects (Murata et al., 1997; Yokoyama et al., 1998a,b,c). At the time of their past admission to the hospital, their plasma cholinesterase had been depressed by about 25 percent of normal. Murata and colleagues (1997) first reported on their responses to sensory evoked potentials, a noninvasive method of detecting functional activity elicited by stimulation of specific nerve pathways, however any functional changes by EEG do not indicate their pathological basis. The event-related potential (ERP) (P300) and the visual-evoked potential (VEP) (P100) displayed slight yet significant prolongation in sarin-exposed subjects, compared with 18 sex- and age-matched control subjects (healthy volunteers).[13] There was no relationship in the sarin-exposed group between neurophysiological findings and scores for PTSD, which were significantly elevated compared to controls (Yokoyama et al., 1998c) Short-latency brain stem auditory evoked potentials and electrocardiography were not different between cases and controls. Findings were interpreted by the authors as suggestive of long-term neurotoxic effects of high-level exposure to sarin in those individuals who no longer reported symptoms.

The same sarin-exposed individuals underwent neurobehavioral testing and vestibulocerebellar testing (Yokoyama et al., 1998a,b). For neurobehavioral testing, cases and controls filled out a PTSD checklist and underwent nine tests: digit symbol (psychomotor performance); picture completion (visual perception); digit span (attention and memory); finger tapping (psychomotor performance); reaction time (psychomotor performance); continuous performance test (sustained visual attention); paired-associate learning (learning and memory); General Health Questionnaire (psychiatric symptoms); and the Profile of Mood States. The score on the digit symbol test for sarin-exposed cases was significantly lower than for controls. The scores on the General Health Questionnaire, fatigue (Profile of Mood States), and PTSD checklist were significantly higher for the sarin group. Their scores on the digit symbol test remained significantly decreased even after controlling for the effect of PTSD. It is important to control for PTSD because studies of military trainees under mock defensive chemical

[13]In the ERP test, subjects' EEG was measured in response to a random sequence of tones. In the visual-evoked potential, their EEG was measured after stimulation with a checkerboard pattern, which reversed at a rate of two times per second. P300 and P100 refer to the peak electrical potential recorded by the EEG.

warfare conditions revealed that 10–20 percent reported (in the absence of actual exposure to chemical weapons) moderate to severe psychological symptoms, including anxiety, claustrophobia, and panic (Fullerton and Ursano, 1990).

For vestibulocerebellar testing, Yokoyama and colleagues (1998a) used computerized posturography on sarin cases and controls. Computerized posturography is a standard means of assessing vestibular function by placing subjects in the middle of a platform and measuring how their movements displaced the platform (via pressure transducers connected from the platform to a computer). The study found significant impairment only in female cases (n = 9) who performed more poorly (with their eyes open) in their ability to maintain postural sway and their center of gravity when they moved at low frequencies (0–1 Hz) in the anterior–posterior direction. Female patients also performed more poorly in the area of sway (i.e., the area on the platform over which the test subject moves to maintain balance). None of the postural sway tests were abnormal in male cases (n = 9). The authors viewed their findings as suggestive of a gender difference in a "delayed" effect of acute sarin poisoning on the vestibulocerebellar system. Their characterization of this effect as "delayed" is questionable, since there is no evidence of this postural testing having been performed at an earlier point after sarin exposure. Thus, the effect may be chronic, rather than delayed.

The Tokyo sarin experience confirms that acute exposure to sarin leads to the acute cholinergic syndrome. Sarin exposure at high levels can be fatal if cardiopulmonary compromise or convulsions ensue. Visual disturbances are frequent sequelae of the acute exposure, particularly in individuals with high-level exposure. Neurophysiological testing of a small group of asymptomatic sarin-exposed individuals does show chronic changes in visual and event-related evoked potentials and vestibulocerebellar function months after the acute syndrome has subsided. These neurophysiological data are suggestive of subtle, persistent CNS effects from sarin. Except for digit symbol test abnormalities, significant cognitive deficits were not detected.

Gulf War Veterans

As explained earlier in this chapter, CIA–DoD modeling determined that U.S. troops located within 25 km of the Khamisiyah weapons site demolition in March 1991 may have been exposed to low or intermediate levels of sarin (CIA–DoD, 1997). U.S. troops did not report acute cholinergic symptoms at the time, but the possibility of low-level, asymptomatic exposures cannot be discounted. In a series of studies on members of a naval battalion (n = 249) 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[14] were more

[14]Based on self-reports about their perceptions of CW exposure, rather than any evidence of symptomatology. Their geographical and temporal location in relation to the Khamisiyah demolition site was not reported. The questionnaire was sent to participants in 1994, before DoD reported that chemical weapons exposure could have occurred.

likely to be classified as having one of six new proposed syndromes (Haley et al., 1997; see also Chapter 2). Specifically, this syndrome—labeled by the investigators as "confusion–ataxia" or "syndrome 2"—features problems with thinking, disorientation, balance disturbances, vertigo, and impotence. This was the only syndrome of the six to have been associated with self-reported chemical weapons exposure (see Chapter 6).

A follow-up study of vestibular function was performed on a subset of those veterans (n = 23) 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. Of the 23 veterans in this study, 13 exhibited syndrome 2, whereas the others exhibited syndromes 1 (impaired cognition) and 3 (arthromyoneuropathy) (see Chapter 2). Based on a new questionnaire, veterans with syndrome 2 reported dizzy spells with greater frequency and longer duration than veterans with the other two syndromes. Veterans with syndrome 3, but not syndrome 2, performed significantly differently from controls on dynamic platform posturography (a test similar to that used by Japanese researchers to identify impairment in sarin-exposed females; see Yokoyama et al., 1998a). Veterans with other syndromes also had performance decrements on some of the measures of vestibular function. The study concluded that there was both 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 these newfound chronic syndromes represent variants of OPIDN caused by exposure to various combinations of organophosphates (pesticides and nerve agents) and carbamate pesticides that inhibit cholinesterases and NTE (see Chapters 2 and 6).

Genetic Susceptibility to Sarin Toxicity

One of the mechanisms of sarin inactivation is by hydrolysis with the enzyme paraoxonase (PON1), an esterase found in liver and serum. The human PON1 gene has polymorphisms at positions 192 (*Arg/Gln*) and 55 (*Leu/Met*) (Furlong et al., 1993). The former accounts for three genotypes (QQ, RR, and QR) relating to the catalytic properties of two forms of an enzyme (types R and Q allozymes), which hydrolyze certain organophosphates at different rates. The R allozyme (Arg_{192}) hydrolyzes the organophosphate paraoxon at a high rate; however, it has a low activity toward 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, on the other hand, has high activity toward organophosphate nerve agents (and low activity toward paraoxon). Thus, individuals with the Q allozyme (QQ or QR) are expected to have greater hydrolysis of sarin than individuals homozygous for the R allele (RR). Since hydrolytic activity with the same genotype can vary about tenfold, it is also important to determine the level of allozyme expression—in addition to

the genotype—in order to characterize an individual's PON1 status (Richter and Furlong, 1999). In Caucasian populations, the frequency of the R allele is about 0.3, but the frequency is 0.66 in the Japanese population (Yamasaki et al., 1997). This would make individuals in the Japanese population more sensitive to the toxicity of sarin, a fact that may have contributed to their morbidity and mortality after the terrorist attacks.

A recent study investigated PON1 genotype and serum enzyme activity in a group of 25 ill Gulf War veterans and 20 controls (Haley et al., 1999). Ill veterans were more likely than controls to possess the R allele (QR heterozygotes or R homozygotes) and to exhibit lower enzyme activity. This study raises the possibility that the R genotype (low sarin-hydrolyzing activity) may represent a risk factor for illness in Gulf War veterans. However, because of the very small size of the study, such findings necessitate further confirmation in a larger population (Furlong, 2000) (also see Chapter 6).

CONCLUSIONS

The committee reached the following conclusions after reviewing the literature on sarin. The committee was unable to formulate any conclusions about cyclosarin because of the paucity of toxicological and human studies.

The committee concludes that 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. This syndrome, as well as cholinergic signs and symptoms, is evident seconds to hours after exposure (see Table 5.2) 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.

The committee concludes that 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 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; and 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.

These conclusions are based on retrospective studies of three different exposed populations in which the acute cholinergic signs and symptoms were documented as an acute effect of exposure. The findings from those studies are based on comparisons with control populations. One population consisted of industrial workers accidentally exposed to sarin in the United States; the other two populations were 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 a maximum of 3 years, depending on the study. Whether the health effects noted above persist beyond the 3 years has not been studied.

The committee concludes 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 health effects.

On the basis of positive findings in a study of nonhuman primates and in studies of humans exposed to organophosphate insecticides (see Appendix E), it is reasonable to hypothesize the occurrence of long-term adverse health effects from exposure to low levels of sarin. Studies of low-level exposure of workers find that organophosphate insecticides are consistently associated with higher prevalence of neurological and/or psychiatric symptom reporting (see Appendix E). However, there are no well-controlled human studies expressly of sarin's long-term health effects at doses that do not produce acute signs and symptoms.

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CONTENTS

6

Pyridostigmine Bromide

Pyridostigmine bromide (PB) is a drug used during the Gulf War as a pre-treatment to protect troops from the harmful effects of nerve agents. It has been used for more than 40 years in the routine treatment of myasthenia gravis and may be used following surgery in the reversal of neuromuscular blockade (Williams, 1984).

PB, a reversible cholinesterase (ChE) inhibitor, is a carbamate compound, specifically, the dimethylcarbamate ester of 3-hydroxy-1-methylpyridinium bromide. It was synthesized in 1945 by Hoffman-La Roche Laboratories in Switzerland and is sold under the trade name Mestinon bromide (Williams, 1984). PB is one of the quaternary ammonium anticholinesterase compounds, which generally do not penetrate cell membranes. Compounds in this category are poorly absorbed from the gastrointestinal tract and are excluded by the blood–brain barrier (Williams, 1984; Goodman et al., 1996).

Mestinon was approved by the Food and Drug Administration (FDA) in 1955 as safe for the treatment of myasthenia gravis.[1] The FDA also approved an injectable form known as Regenol for reversing the effects of some anesthetic formulations (Rettig, 1999). In the treatment of myasthenia gravis, the average oral dose is 600 mg per day in divided doses; however, the size and frequency of the dose must be adjusted to the needs of the individual patient (*Physicians' Desk Reference*, 2000). The drug is poorly absorbed after oral administration

[1]Myasthenia gravis is an autoimmune disorder characterized by antibody blockade of the acetylcholine (ACh) receptor at the neuromuscular junction.

and peak plasma levels occur at 2 to 3 hours after oral dosing. The drug is eliminated almost exclusively via the kidneys in the urine (Williams, 1984).

Side effects of PB are generally related to the large doses given to myasthenics; in surgical patients, adverse reactions are controlled by simultaneous administration of atropine (Williams, 1984). Adverse reactions may be muscarinic or nicotinic (also see Chapter 5), both reactions are due to increased acetylcholine (ACh). Muscarinic reactions include nausea, vomiting, diarrhea, abdominal cramps, increased peristalsis, increased salivation, increased bronchial secretions, miosis, and heavy perspiration. Nicotinic effects are chiefly muscle cramps, fasciculations, and weakness (Williams, 1984).

During the Gulf War, PB was used as a pretreatment for possible exposure to nerve agents because of its ability to reversibly bind to acetylcholinesterase (AChE).[2] The bound fraction is thereby protected from subsequent exposure to nerve agents that would irreversibly bind to AChE. PB is not an antidote (i.e., it has no value when administered after nerve agent exposure) and is not a substitute for atropine or 2-pralidoxime chloride; rather, it enhances their efficacy (Madsen, 1998).

PB was used as an investigational product during the Gulf War (Rettig, 1999) and was not recommended for routine use. The FDA, under a then newly enacted interim rule, had granted DoD a waiver from the requirement to obtain informed consent from service members taking this drug, but the rule did not address the record keeping that would ordinarily accompany the use of an investigational drug (FDA, 1990; Rettig, 1999). PB was manufactured for Duphar and Roche; it was produced by two different facilities outside the United States, specifically in the Netherlands for Duphar and in the United Kingdom for Roche.

The Department of Defense (DoD) reported that 5,328,710 doses were fielded and estimated that approximately 250,000 personnel took at least some PB during the Gulf War.[3] It was supplied as a 21-tablet blister pack, the dosage prescribed was one 30-mg tablet every 8 hours.[4] Variation in use occurred, how-

[2]AChE is an enzyme necessary to remove ACh. Acetylcholine transmits nerve signals at the cholinergic neuromuscular junction or synapses in the central nervous system. Anticholinesterase agents inhibit (inactivate) AChE, resulting in an accumulation of ACh. The accumulation repetitively activates the ACh receptors, resulting in exaggerated responses of the organ (e.g., excess salivation).

[3]The number of doses fielded was obtained through a search of the Defense Personnel Support Center archived logistic records for Operations Desert Shield and Desert Storm and reflects the amount of product ordered and sent through supply channels. In most cases, only a review of each individual's medical treatment record would provide the actual number of doses administered, and few records were maintained by individuals (Office of the Secretary of Defense Bernard Roskter, January 30, 1998 letter to the Honorable Arlen Specter, Chairman, Committee on Veterans' Affairs, U.S. Senate, 1998).

[4]PB was distributed as 30-mg tablets in a blister pack of 21 tablets within a sealed pouch. Each packet provided a 1-week supply of PB for one person. Military personnel were issued two blister packs each. Recommended long-term storage was at 2–80°C, and blister packs removed from refrigeration were to be used within 6 months (Madsen, 1998).

ever, because it was self-administered and to be taken only when ordered by the unit commander (PAC, 1996). Thus, actual veterans' exposures to PB are not known, and there are few examples of documentation in either individual health records or unit records, making it difficult to assess any potential contribution of this drug to the current unexplained illnesses (PAC, 1996).

DoD noted that at the recommended dosage levels, soldiers reported acute but transient side effects. Keeler and colleagues (1991) conducted an uncontrolled retrospective survey of the medical officers of the XXVIII Airborne Corps. The unit's 41,650 soldiers were instructed to take PB at the onset of Operation Desert Storm in January 1991. Usage varied from 1 to 21 tablets taken over 1–7 days; 34,000 soldiers reported taking the medication for 6–7 days. Reported side effects of PB were estimated to have been present in half the troops; they were not incapacitating, however, and were primarily gastrointestinal in nature. An estimated 1 percent of the soldiers believed they had symptoms that warranted medical attention, but less than 0.1 percent had effects sufficient to warrant discontinuation of the drug (Keeler et al., 1991).

PB, alone and in combination with other exposures, has been suggested as one of several possible causative factors associated with illnesses in Gulf War veterans (Abou-Donia et al., 1996a,b; Chaney et al., 1997; Fukuda et al., 1998; Unwin et al., 1999). The remainder of this chapter examines the scientific literature on the potential adverse health effects of PB.

The committee begins its review with a discussion of the toxicology and pharmacokinetics of PB, based primarily on findings from animal studies and other experimental test systems. The committee then turns its attention to studies in humans. These include clinical studies, related principally to the use of PB in the treatment of myasthenia gravis and its use as a test of hypothalamic pituitary function or growth hormone response. In addition to these clinical studies, the committee reviewed studies in healthy volunteers and epidemiologic studies. The healthy-volunteer studies were conducted among healthy military and nonmilitary populations to evaluate the tolerance of prophylactic doses of PB. Unfortunately, there is a paucity of epidemiologic studies on PB and adverse health effects in the peer-reviewed literature. Although there have been a number of descriptive epidemiologic studies of Gulf War veterans (see Chapter 2), those investigations generally sought to characterize the nature and frequency of the symptoms and illnesses reported by returning soldiers and did not examine the association of PB with the illnesses reported. Studies that attempted to evaluate the association of PB and symptoms among Gulf War veterans are reviewed.

TOXICOLOGY

There is an extensive toxicologic literature on PB, which was reviewed by the committee. The studies discussed below were designed to assess the pharmacologic and toxic properties of PB in animals and other test systems.

Structure and Mechanism of Action

There are several types of esterases in the body, all of which hydrolyze esters such as acetlycholine. Some of those in the plasma are nonspecific and hydrolyze many esters including ACh, whereas acetylcholinesterase, which is found at cholinergic synapses, is more specific for ACh. AChE is also found in erythrocytes, but its function in these cells is poorly understood. Inhibition of the plasma (or erythrocyte) esterase is without known consequence, whereas the inhibition of AChE at cholinergic synapses leads to a spectrum of toxicological effects.

At cholinergic synapses, ACh released from nerve endings by action potentials activates the postjunctional receptors and thereby elicits responses. To prevent it from inappropriately reactivating the receptors, ACh is hydrolyzed to inactive products by the enzyme AChE in the synapse, thus ensuring that one action potential leads to a single response. Interference with the ability of AChE to hydrolyze ACh leads to accumulation of the latter in the synapse, and the excess neurotransmitter is then responsible for both the pharmacological and the toxicological manifestations of AChE inhibition.

The toxicokinetics of PB are complex, and there is incomplete agreement on the fate of an ingested dose (Joiner and Kluwe, 1988; Golomb, 1999). The gastrointestinal tract erratically absorbs PB, leading to considerable variations in plasma concentration (Aquilonius et al., 1980). Absorbed PB is subject to first-pass metabolism by the liver (Barber et al., 1975), but since 60–85 percent of an administered dose is excreted unchanged via the kidney, the fraction of a dose undergoing hepatic biotransformation is not large. Hepatic biotransformation of neostigmine and pyridostigmine apparently gives rise to the metabolites 3-hydroxy-*N*-methylpyridinium, 3-hydroxyphenyltrimethylammonium, and edrophonium (Hennis et al., 1984); there is no evidence that these metabolites contribute to antagonism of neuromuscular blockade or that they are neurotoxic.

The differences in duration and reversibility of cholinesterase inhibition by PB and organophosphate exposures provide the rationale for battlefield use of PB by the military. Although both PB and the organophosphate (OP) compounds employed as "nerve gases" inhibit AChE by binding to it, the OP–AChE bond is much stronger than the PB–AChE bond, making the former essentially irreversible. The differences in binding of carbamates and organophosphates to AChE have been exploited in the use of a reversible inhibitor of AChE (e.g., PB) to protect it against irreversible inhibitors such as the nerve gases (Gordon et al., 1978; Dirnhuber et al., 1979). In effect, protection results from "pre-inhibition" of the enzyme with a more readily reversible inhibitor.

As noted, the prophylactic use of PB in military personnel calls for 30 mg to be taken three times a day. Since the plasma half-lives of orally administered PB are 120–195 minutes and the corresponding half-lives for reversal of erythrocyte AChE inhibition are in the same range (Kluwe et al., 1990), 8-hour intervals between doses are adequate to maintain constant levels of AChE inhibition and thus protection. Joiner and Kluwe (1988) found 30 percent inhibition of red blood cell (RBC) AChE in monkeys following oral administration of 0.28 mg/kg

PB: higher doses caused proportionally greater inhibition (0.57 mg/kg yielded 43 percent inhibition).

Animal Studies

Many neural and neuromuscular systems in the body employ ACh as a neurotransmitter, and still other organs are influenced by ACh. Given the numerous physiological functions influenced by ACh, it is not surprising that perturbations of its function, resulting from AChE inhibition by PB, have numerous and diverse toxicological consequences. There are many recently published and ongoing studies that may elucidate the nature of the mechanisms of PB toxicity; however, many of the studies have yet to be confirmed. The following sections briefly review the available information on PB toxicity, including those studies that await replication.

Neuromuscular Effects

The neuromuscular effects of PB are important for two reasons. First, impairment of neuromuscular function leads to muscle weakness. Second, experimental findings obtained from the readily accessible neuromuscular junction have long been interpreted to be applicable to other cholinergically innervated synapses in the central nervous system, which are much more difficult to access experimentally. Thus, events occurring at the neuromuscular junction have been thought to mirror those in the brain.

PB, as a ChE inhibitor, modifies physiological function at the sites of innervation of all types of muscle: smooth, cardiac, and skeletal (or striated). The neuromuscular effects of PB have been described almost exclusively for skeletal muscle, while those in other types of muscle are relatively less studied. The effects of PB on the skeletal neuromuscular junction have recently been reviewed in detail (Golomb, 1999). Effects of PB on cardiac muscle have been reported (Glass-Marmor et al., 1996).

Exposure to PB has pharmacological and/or toxicological consequences on neuromuscular function either by direct action of PB at low doses, acting as a weak agonist at the nicotinic ACh receptors (Sherby et al., 1984; Maelicke et al., 1993), or more importantly by accumulation of ACh resulting from inhibition of AChE. Acutely, PB leads to a facilitation (or augmentation) of the strength of contractile tension developed in skeletal muscle because ACh accumulation repetitively activates the contractile process. The relationship between the degree of ChE inhibition and the facilitation of twitch tension is complex. No twitch potentiation is seen until RBC AChE is at least 85 percent inhibited (Barber et al., 1979); this threshold is nearly identical to that noted for other ChE inhibitors. At inhibition levels of 85–98 percent there is a linear relationship between AChE inhibition and facilitation of twitch tension. Large doses of PB would normally be required to achieve these levels of RBC AChE inhibition, and it

would be anticipated that numerous other incapacitating consequences of ChE inhibition, particularly muscarinic (e.g., salivation, sweating), would be apparent before neuromuscular effects became manifest.

Failure of neuromuscular transmission, whereby nerve signals no longer evoke muscle contraction, is also thought to be an extension of the effects of accumulation of ACh. Prolonged depolarization leads to a desensitization of the postjunctional receptor; high doses of ChE inhibitors may further directly block ACh receptors, adding to the desensitization (Maselli and Leung, 1993a,b). Neuromuscular blockade by this mechanism would require very large doses of PB.

It has long been known that inhibition of AChE at the neuromuscular junction results in both pre- and postjunctional morphological alterations, and the effects of PB exposure are no exception (Hudson et al., 1986; Matthew et al., 1998). Alterations in the prejunctional apparatus of the neuromuscular junction (i.e., the nerve ending), most often associated with denervation phenomena, are not usual sequelae of PB intoxication; rather most evidence of exposure occurs postsynaptically. Microscopic examination of the postsynaptic and myofibrillar structures following exposure to PB reveals that most damage occurs in the vicinity of the neuromuscular junction; Z-lines are blurred and electron microscopy reveals swollen mitochondria, suggestive of a disruption in calcium homeostasis (Gebbers et al., 1986). Myopathic changes decrease with distance from the postjunctional region (Adler et al., 1992), and normal myofibers occur within distances of 12–14 microns. Studies in which myopathic changes were observed employed large doses of PB (20–98 mg/kg per day), which yielded inhibition of AChE in excess of 50 percent (Hudson et al., 1985; Bowman et al., 1989**)**, greater than the inhibition seen in humans following PB administration. When blood ChE inhibition was reduced to levels expected (about 30 percent) by reducing the PB dose, neither acute nor subchronic (4-week) exposure produced neuromuscular lesions (Matthew et al., 1998).

The susceptibility of neuromuscular junctions to neural and/or myofibrillar damage does not appear related to fiber type, being observed in muscles with substantially different fiber type compositions (Hudson et al., 1985). Despite the initial appearance of pathological alterations at the neuromuscular junction during continuous administration of PB, these alterations (principally myopathic) reversed by the second week of daily exposure to 90 mg of PB (Bowman et al., 1989; Matthew et al., 1990). Similar patterns of myopathic lesions (i.e., initial appearance of lesions which subsequently resolve) are observed with exposure to other carbamate and to organophosphorus ChE inhibitors (reviewed recently, Golomb, 1999). Acetylcholine-associated myopathy is not a new observation (Fenichel et al., 1974).

All ChE inhibitors cause cholinergic toxicity as a result of the accumulation of excess amounts of ACh; hence they induce similar toxicities (generally referred to as acute toxic or cholinergic effects). In addition to acute toxicity, certain ChE inhibitors, particularly the organophosphorus compounds, produce other neuro- and myopathic effects, which are apparently unrelated to ChE inhibition and are described as intermediate and delayed neurotoxicity (or organo-

phosphate-induced delayed neuropathy [OPIDN]). Intermediate syndrome (intermediate in onset between the acute toxic effects following exposure to a ChE inhibitor and the delayed neuropathic actions associated with certain OP-type cholinesterase inhibition) is a toxic syndrome associated with muscle weakness. It typically occurs 24 hours after exposure and is characterized by weakness or paralysis involving neck flexors, cranial nerves, proximal limb muscles, and respiratory muscles (Leon et al., 1996). The intermediate syndrome usually resolves over time, and although it has been associated with exposure to a variety of ChE inhibitors, PB has not been implicated (Golomb, 1999). Clinically, OPIDN (see below and Chapter 5) is a delayed neuropathy, its symptoms becoming manifest some 2–3 weeks after exposure to certain organophosphate ChE inhibitors. A detailed description of the disorder has been given recently (Golomb, 1999). Like the intermediate syndrome, OPIDN has not been associated with exposure to PB.

PB administration also results in enhanced expression of AChE in skeletal muscle, evident even after the enzyme is no longer inhibited (Lintern et al., 1997a,b). Further, repeated administration of PB over a period of weeks produces a dose-related increase in the expression of beta-endorphin and beta-endorphin 30-31 (glycylglutamine), both of which are derived from the same precursor protein pro-opiomelanocortin (POMC); the endorphins are thought to lead to the augmented AChE (Amos and Smith, 1998). POMC levels are also increased by nerve section (Edwards et al., 1986), as well as by other neurotoxicants including iminodipropionitrile (IDPN), acrylamide, and organophosphates (Hughes et al., 1992, 1995; Amos and Smith, 1998). Thus, increased expression of POMC and the consequent increase in AChE levels are probably obligatory components of an injury response, regardless of whether the injury is physical or chemical.

Neurobehavioral Toxicology

Compared to other carbamates, particularly physostigmine, or to organophosphate cholinesterase inhibitors, PB has had limited investigation for its potential neurobehavioral effects. Based on its reported lack of access to the central nervous system (CNS), PB has historically been used as a negative control in behavioral studies of other ChE inhibitors or as an agent to selectively produce peripheral nervous system actions of ChE inhibitors. PB is a carbamate possessing a positively charged quaternary group that restricts its penetration of the blood–brain barrier (Xia et al., 1981). Doses of PB employed in these studies, often in the range of 200 μg/kg, failed to produce observable effects on the behavioral paradigm under examination (McMaster and Finger, 1989; Wolthius et al., 1995); thus, PB has traditionally been thought to be devoid of CNS action.

The use of PB as a preventive measure against the effects of chemical warfare agents, coupled with the emerging understanding of the importance of a cholinergic link in Alzheimer's disease, has led to a reexamination of the action of PB, particularly of chronic dosing schedules, on behaviors in both humans and laboratory animals. Low doses of PB have been reported to have behavioral

consequences after acute administration. Two-way shuttle box avoidance learning, open-field behavior, and complex coordinated movements in rats were interrupted by PB at doses of about 0.27 mg/kg, which neither produced overt symptoms nor affected running speed and coordinated locomotion (Wolthius and Vanwersch, 1984). Shih and colleagues (1991) examined a wider range of PB doses on lever pressing of rats maintained under a multiple fixed-ratio (FR 20) time-out schedule of reinforcement for water reward. They noted that doses greater than 6 mg/kg disrupted responding but there were no overt signs of peripheral neurotoxicity until doses in excess of 12 mg/kg were administered. Liu (1991) confirmed that doses of 3–12 mg/kg interfered with responding but did not cause overt toxicity.

PB has been tested in primates (*Macaca mulatta*) for its effects on the ability of subjects to perform compensatory tracking on an equilibrium platform (Blick et al., 1994). Of the doses of PB tested, only the highest dose interfered with performance of the task (Murphy et al., 1989). Plasma ChE inhibition at this dose was 83 percent. Thus, it appears that PB, particularly at higher doses, is capable of modifying experimentally measured behavioral end points. This might suggest some degree of entry of PB into the CNS.

Gastrointestinal Effects

Many aspects of gastrointestinal function are mediated or influenced by ACh, and PB would be predicted to cause gastrointestinal disturbance, especially if administered orally. Thus, the most common complaints of troops taking the prescribed dosage of PB (3 × 30 mg per day—the equivalent of 0.4 mg/kg every 8 hours) included nausea, vomiting, diarrhea, abdominal cramping, increased salivation, bronchial secretions, miosis, and diaphoresis—symptoms referable to a parasympathetic (and peripheral) predominance. Human symptoms are in accord with observations made in laboratory animals. In beagles, the threshold dosage for gastrointestinal effects of PB is as low as 0.05 mg/kg; this dose causes significant inhibition of both plasma butyrylcholinesterase and RBC AChE (Kluwe et al., 1990). Higher doses of PB result in proportionally greater effects. Species differences in responsiveness to toxicities of PB have been noted (Levine et al., 1991).

180-Day Exposure

One 180-day subchronic oral toxicity study of PB has been reported (Morgan et al., 1990). Rats were administered 0–10 mg PB/kg, 5–7 days per week, for 180 days. During the dosing phase of the study, ChE inhibition was up to 63 percent in plasma and 49 percent in erythrocytes. An extensive battery of tests (including hematological and serum analyses) were performed 30 days after the last dose of PB, at which time ChE levels had returned to control values. Although serum chemistry revealed elevations in lactate dehydrogenase,

creatine phosphokinase, and aspartate aminotransferase, these indices of myopathy (Hoffman et al., 1989) were unaccompanied by morphological evidence of PB-induced toxicity.

Reproductive Effects

A single study in rats has been reviewed that reports the reproductive toxicity of PB (Levine et al., 1989; Levine and Parker, 1991). No teratogenic effects were noted even after 90 days' exposure to doses ranging up to 60 mg/kg per day. Although there was a suggestion of postimplantation loss at the highest dose tested (which also resulted in 10 percent mortality), other fertility indices and offspring were unchanged in perinatal and postnatal studies.

Contact Dermatitis

In skin sensitization studies, 44 percent of guinea pigs exposed to 50 percent PB alone exhibited positive responses. Addition of dermal penetration enhancers (surfactants) such as sodium lauryl sulfate increased the incidence of positive responses to greater than 80 percent (Harris and Maibach, 1989).

Bronchial Asthma

Excess ACh resulting from ChE inhibition might be expected to exacerbate bronchial asthma by causing increased respiratory secretions and bronchoconstriction. Dogs administered PB doses of 2–5 mg/kg exhibited dose-dependent increases in airway resistance and decreases in tidal volume (Caldwell et al., 1989). Since these doses are much higher than those given to humans, no complications of PB administration in asthmatics were predicted. However, there have been reports of human studies (discussed later in this chapter) and anecdotal reports suggesting a possible dose-dependent outcome in asthmatics (Ram et al., 1991; Gouge et al., 1994).

Cardiac Dysrhythmias and Cardiomyopathy

The autonomic, parasympathetic innervation of the heart is concerned principally with the regulation of heart rate and atrioventricular conduction and exerts this influence via cholinergic synapses much like those found elsewhere in the nervous system. Inhibition of AChE at these synapses results in prolonged residence time of ACh, leading to slowing of the sinoatrial firing rate (bradycardia), along with prolongation of phase four conduction parameters. Recent studies in cats indicate that the magnitude of the bradycardia resulting from PB does not correlate with the degree of AChE inhibition, but rather reflects the extent of muscarinic agonist actions (Yamamoto et al., 1996; Stein et al., 1997).

Scattered reports (Kato et al., 1989; Glass-Marmor et al., 1996) suggest that ACh accumulation in cardiac muscle compromises mitochondrial function and thus impairs myocardial energetics in a manner similar to that observed in skeletal muscle (see discussion of neuromuscular effects). The doses of PB employed in these studies were 20 and 60 mg/kg, respectively.

Thermoregulation

Like other ChE inhibitors, PB is capable of altering cholinergically mediated thermoregulatory processes in the hypothalamus. The cholinergic component is demonstrated by the ability of atropine to block PB alterations in thermoregulation (Matthew et al., 1988). Compromised temperature regulation is most prominent at higher ambient temperatures. In rats, acute administration of PB produces hyperthermia, whereas chronic administration elicits much less elevation in body temperature, indicating the relatively rapid emergence of adaptive processes akin to heat acclimation during prolonged exposure (Matthew et al., 1994). In contrast, mice given 0.2 mg/kg PB have been reported to be hypothermic (Kaufer et al., 1999). It appears that hyperthermia (and the extent of debilitation) is correlated with the degree of brain ChE inhibition (and presumably with the extent of penetration of the ChE inhibitor through the blood–brain barrier) and, further, there are no significant effects on temperature regulation when plasma ChE inhibition is less than 30 percent (Francesconi et al. 1984, 1986; Matthew et al., 1988, 1994). This also appears to be true for humans exposed to PB (Seidman and Epstein, 1989).

The impact of physical conditions such as heat, alone and in combination with pharmacological agents, on task performance has been evaluated in both animals and military personnel. PB is of particular interest in this regard since sweating is under autonomic, muscarinic control. In monkeys, doses of PB that produce a 25–30 percent inhibition of serum ChE levels result in only transient alterations in physiological parameters (Avlonitou and Elizondo, 1988). Francesconi and colleagues (1986) reported that chronic (14-day) inhibition of ChE in rats to levels as high as 39 percent is without effect on thermoregulation or exercise performance. In human volunteers, single doses of 30 mg are without effect on psychomotor performance or thermoregulation (Wenger and Latzka, 1992) as were multiple doses (Seidman and Epstein, 1989; Izraeli et al., 1990; Arad et al., 1992a,b). Chronic administration of PB does not appear to alter thermoregulation at cold ambient temperatures (Sawka et al., 1994).

It has been reported (Sharma et al., 1992) that moderate heat stress (38°C for 4 hours) enhances the entry of tracers such as Evans blue into the brains of rats, adding to earlier evidence supporting the notion that stress augments the permeability of the blood–brain barrier (Belova and Jonsson, 1982; Ben-Nathan et al., 1991). Subsequent studies have failed to confirm these findings. Lallement and colleagues (1998) administered tritiated PB to guinea pigs maintained at an ambient temperature of 42.6°C for 2 hours and noted that even though those given PB succumbed to heat stress and exhibited high levels of plasma

cortisol (an indicator of stress), there was no inhibition of brain AChE. There was also no autoradiographic evidence that PB had entered the CNS. Hence, stress in guinea pigs fails to enhance PB penetration, while in mice (Friedman et al., 1996) and rats (Sharma et al., 1992), stress appears to permit entry of the drug. Whether these differences stem from species differences, age of the animals used, or other variables remains to be determined.

Interactions with Other Agents

Interactions between agents present during the Gulf War have been hypothesized or suspected to contribute to the illnesses of Gulf War veterans (IOM, 1995; PAC, 1996, 1997). Several mechanisms exist whereby other chemical compounds may influence the pharmacological and toxicological actions of PB. These may occur through pharmacological antagonism, synergism, addition, and so forth, presumably by actions on entirely different receptor types. Alternatively, the presence of other chemical(s) may enhance absorption or interfere with detoxification processes, leading in either case to an exaggeration of the pharmacological and/or toxicological effects of PB. The possible outcomes of the interactions of several relevant chemicals have recently been discussed (Golomb, 1999).

Pharmacological Interactions

There have been limited studies of possible toxic interactions between PB and other agents to which there was reported or putative exposure during the Gulf War. Co-exposure of hens to total cumulative doses of 200, 400, and 20,000 mg/kg of PB, chlorpyrifos, and *N*,*N*-diethyl-*m*-toluamide (DEET) respectively, over 2 months resulted in increased indices of toxicity (Abou-Donia et al., 1996a). Neurological indices of dysfunction were more severe in birds receiving the combined exposures, paralleling perhaps the more prominent neuropathology observed in the sciatic nerve and spinal cord and the greater degree of inhibition of plasma ChE and brain AChE. It is noteworthy that the pathology and the neurological impairment are hallmarks of OPIDN, but symptoms of this neurotoxic disorder are not consistent with those reported in Gulf War veterans' illnesses. Also, in this study (Abou-Donia et al., 1996a), neither PB nor DEET inhibited NTE (neuropathy target esterase), consistent with observations that neither produces OPIDN. Chlorpyrifos, an organophosphate pesticide used in the Gulf War, does inhibit NTE, but only by 27–29 percent, which is below the threshold of inhibition required to precipitate OPIDN.

An analogous study by the same authors (Abou-Donia et al., 1996b) exposed hens to combinations of PB and DEET, but with administration of permethrin (total dose 20,000 mg/kg) rather than chloropyrifos. Again, combinations of the agents proved more toxic than single exposures, and with the substitution of permethrin for chlorpyrifos, weaker inhibition of ChE was noted.

The authors speculated that the enhanced toxicity may result from interference with detoxification processes and raised the possibility of polymorphisms in esterases that play a determining role in toxic outcomes. Similarly, combinations of very large doses of PB, DEET and permethrin have been reported to increase the lethality of these agents in rats (McCain et al., 1997). The use of such large dosage levels in these studies makes interpretation of possible toxic synergisms problematic. A number of hypothetical mechanisms of interactions have been offered (Golomb, 1999).

There is no a priori reason to suspect that PB is capable of causing OPIDN. PB is not known to inhibit NTE, a hallmark of chemicals causing OPIDN. Although PB is known to inhibit butyrylcholinesterase (BuChE), it does not appear to be a substrate for paraoxonase;[5] hence it seems biologically implausible that there is a genetic susceptibility for OPIDN to be induced by PB via this mechanism. Further, there are insufficient data to determine whether exposure to other chemicals, either before or after PB, enhances its potential to produce delayed neurotoxicity.

There are reports of other classes of chemicals interacting with PB to modify its toxicity. Chaney et al. (1997, 1998) noted that adrenergic agents varied in their ability to potentiate the toxicity of PB without apparent structure–activity relationships. The toxicity of PB was enhanced by certain beta-receptor agonists, and also by some alpha-receptor antagonists, whereas other adrenergic agents were without effect. Catecholamines (epinephrine and norepinephrine) were reported to be additive with PB toxicity. Both potentiation and addition were blocked by atropine, clearly pointing to the cholinergic link in this complex toxicity. In another study, the same authors (Chaney et al., 1999) noted that a toxic interaction of PB and DEET resulted in seizures, which—although resistant to conventional anticonvulsant drugs—were blocked by muscarinic antagonists such as atropine.

Keeler (1990) reviewed possible interactions between PB and drugs used in combat anesthesia and predicted the greatest potential for drug interaction to be with the neuromuscular blocking drugs. The author also recognized potential interactions leading to overstimulation of muscarinic receptors and, hence, to unwanted effects such as laryngospasm and bradycardia.

Available studies in which the toxicity of PB, alone or in combination with other chemicals, has been assessed in laboratory animals have been reviewed. Although data derived from laboratory investigations may have general applicability to humans, there are enough differences in toxic responses to the ChE inhibitors as a class to caution against making direct inferences to humans. For instance, although chickens are reported to be highly sensitive to OPIDN, this disorder is difficult to induce in laboratory rats, except at extremely high doses of organophosphates. Thus, although some general principles from laboratory

[5]Paraoxonase is an enzyme which plays a role in reducing the toxicity of organophosphorous compounds.

studies may be useful in determining human toxic responses, it is unsound to assume that toxic outcomes will be consistent across species.

Enhanced Absorption

Buchholz and colleagues (1997) examined the influence of PB on the CNS uptake of permethrin, at doses and durations relevant to human exposure, and found that oral PB actually reduced the amount of permethrin reaching the central nervous system. Such data, although limited, do not support a role for either decreased metabolism or enhanced penetration into the brain as the basis for enhanced toxicity. Little information regarding the influence of PB on absorption of other agents, or vice versa, is available; however, DEET has been used as a transdermal carrier for drug delivery (Hussain and Ritschel, 1988).

Interference with Metabolic Disposition

Simultaneous exposure to multiple therapeutic agents has repeatedly demonstrated the capacity for one drug to interfere with the metabolism of a second agent. This interaction may facilitate or impair biotransformation, the outcome of which is to diminish or exaggerate the pharmacological or toxicological actions of the chemical. Further, both pyrethroids and carbamates are known to rely to some extent on the cytochrome P-450 system for their metabolism, and possibilities exist for interactions to have toxic outcomes (Casida et al., 1983; Eiermann et al., 1993; Selim et al., 1995). However, direct experimental evidence for such interactions is lacking.

Penetration into the Brain

The brain is protected from many classes of xenobiotics by the blood–brain barrier (BBB), a layer of endothelial cells that prevents the movement of many chemicals from the circulation into the brain. Since the nervous system in the periphery is not afforded similar protection, PB would be expected to manifest its pharmacological and toxicological actions primarily in the peripheral nervous system.

The BBB is a specialized structure responsible for the maintenance of the central neuronal microenvironment, playing a pivotal role in tissue homeostasis, fibrinolysis and coagulation, vasotonus regulation, the vascularization of normal and neoplastic tissues, and blood cell activation and migration during physiological and pathological processes. Regulation of blood–tissue exchange is accomplished by individual endothelial cells. A pivotal function of endothelial cells is to regulate the selective transport and metabolism of substances from the blood to the brain. Because of the existence of tight junctions between adjacent endothelial cells, nonspecific paracellular ionic leakage across the BBB is thought to be minimal. It should be pointed out, however, that not all of the CNS

vasculature conforms to the above morphological description. Structural attributes of endothelial cells in nonbarrier areas (i.e., areas that lack the BBB) have been examined most systematically in the circumventricular organs. In contrast to the zonulae occludentes junctions of tight barrier areas, endothelial cells in the circumventricular organs exhibit maculae occludentes junctions, which only partially occlude the gaps between adjacent endothelial cells. Hence, the barrier is not as tight, and diffusion across the capillaries is more prevalent.

From the toxicological point of view, areas that lack a true BBB represent potential sites for the accumulation of neurotoxins, because their passage into the brain parenchyma is likely to be less restrictive. Circumventricular organs that lack the proper BBB are midline structures bordering the third and fourth ventricles. They include the pineal gland, median eminence, subfornical organ, area postrema, subcommissural organ, and organum vasculosum of the lamina terminalis (Aschner, 1998).

Friedman and colleagues (1996) raised the possibility that PB, combined with stress, may have enhanced potential to penetrate the BBB acting via a cholinergic mechanism. Experiments were conducted in which mice were stressed by forcing them to swim, a procedure reported to cause opening of the BBB (Sharma et al., 1991). Penetration of both Evans blue and AChE plasmid DNA including the cytomegalovirus promoter in the brain were increased tenfold in stressed mice, confirming that the procedure effectively produces breaches in the BBB. In stressed mice, the dose of PB required to obtain equivalent inhibition of brain AChE was reduced a hundredfold, suggesting enhanced penetration of PB. Doses of PB up to 1.0 mg/kg did not result in significant inhibition of brain AChE. The enhanced cholinergic stimulation resulting from the now-greater inhibition of ChE induced a cascade of c-Fos-mediated transcriptional responses. Importantly, similar increases in c-Fos mRNA could be elicited within minutes of administration of 2 mg/kg of PB alone.

Because PB is a positively charged carbamate, it is unlikely to gain access to the central nervous system. The observation that the acute symptoms associated with the use of PB are referable to actions on the peripheral nervous system supports this notion. However, the biologic plausibility of some degree of PB penetration into the CNS is suggested by reports of positive actions on behavioral measurements, as well as hypothalamic actions of PB modifying temperature regulation and release of growth hormone (see later discussion). The studies by Friedman and colleagues (1996) are significant in that they provide at least a hypothetical basis for enhanced brain penetration of PB. Although important, the interpretation of these findings must await successful replication and confirmation.

Genetic Susceptibility

Butyrylcholinesterase (BuChE)

Anesthesiologists have long recognized that butyrylcholinesterase exists in more than one variant. A subpopulation of individuals given the neuromuscular-

blocking drug succinylcholine, which relies heavily on BuChE for its hydrolysis, exhibit unexpectedly prolonged paralysis of the respiratory muscles (succinylcholine apnea) because they possess an atypical BuChE. Individuals with atypical BuChE (i.e., aspartate rather than glycine at position 70) (McGuire et al., 1989) are incapable of metabolizing succinylcholine and are also less sensitive to some inhibitors (Neville et al., 1992). Atypical variants of BuChE are encountered in less than 5 percent of the general population, although this may vary in specific subpopulations (Ehrlich et al., 1994).

The possibility has been raised that individuals with atypical BuChE may be more susceptibile to PB toxicity (Loewenstein-Lichtenstein et al., 1995). This suggestion was based on observations of a single patient, homozygous for atypical BuChE, who manifested severe symptoms of toxicity during and immediately following the administration of PB. Since the serum BuChE in this individual was much less susceptible to inhibition by PB (and other carbamate ChE inhibitors), it was suggested that the decreased "buffering capacity" of atypical BuChE would lead to an abnormal accumulation of PB and signs of toxicity.

Hypothetically, the inhibition of plasma esterases by agents such as PB could also represent a loss of capacity to "scavenge" (hydrolyze) other xenobiotics that are esters, leading to their accumulation and toxic consequences (Loewenstein-Lichtenstein et al., 1995; Abou-Donia et al., 1996a,b; Shen, 1998). The possible importance of a scavenger role for BuChE in PB toxicity is suggested by the observation that Wistar rats, which constitutionally have lower levels of BuChE than the Sprague-Dawley strain, also exhibit more exaggerated acoustic startle responses following exposure to PB (Servatius et al., 1998). However, Lotti and Moretto (1995) question the importance of a scavenger role for BuChE as a major contributor to toxicity, noting that even the doses of PB employed in the treatment of myasthenic patients produce little inhibition of BuChE, and further argue that it would be unlikely to play a role in illnesses in Gulf War veterans. Examination of PB-exposed individuals who served in the Gulf War and reportedly are suffering from neurological problems failed to detect any differences between their BuChE activities and those of controls (Haley and Kurt, 1997).

Paraoxonase/Arylesterase 1 (PON1)

Polymorphisms are also known to occur in the PON1 gene. Three genotypes (Q, QR, and R) influence the catalytic activity of two alloenzymes, which—acting as paraoxonases/arylesterases—are capable of hydrolyzing organophosphates at different rates and with differing substrate specificities (Adkins et al., 1993; Humbert et al., 1993; Davies et al., 1996). PON1 activity is known to show considerable variation in humans (Mutch et al., 1992). Haley and colleagues (1999) have recently suggested a relationship between polymorphisms and neurological impairment in Gulf War veterans. The authors identified a study population of veterans with symptom complexes (Haley et al., 1997a,b) and characterized the alleles for both PON1 and BuChE in these individuals (Haley and Kurt, 1997). Among ill veterans, there was a greater tendency to

possess the R allele than in controls, and although the arylesterase activity was somewhat lower, the total paraoxonase activity was higher. Since PON1 does not appear to be involved in the metabolism of PB (Haley et al., 1999) the relationship between this polymorphism and PB toxicity is unclear. The authors suggest that PB, having inhibited BuChE, leaves individuals only PON1 as a defense against organophosphates and that this last line of defense is deficient in genetically predisposed individuals. Although this is an intriguing possibility, direct experimental evidence for a contributory role of polymorphisms in the combined toxicity of PB and organophosphates is lacking and requires additional investigation.

HUMAN STUDIES

This section reviews what is known about the use of PB and potential adverse health outcomes from the literature on patients (clinical studies), healthy volunteers, and epidemiologic studies. In some cases, the patient or healthy-volunteer studies include populations of veterans, as do the epidemiologic studies reviewed by the committee. Several of the studies review general health outcomes, whereas others focus on specific organ systems.

Clinical Studies

There are a large number of clinical studies, principally related to the use of PB as a test of hypothalamic pituitary function or growth hormone response and in the treatment of myasthenia gravis. In addition, a smaller number of clinical studies (i.e., case reports and case series) are available that describe the effects of PB when used in patients. These studies are discussed below.

Clinical Studies of PB and Growth Hormone

Studies have been done on normal subjects and patients with a variety of chronic disorders who were given PB as a test of hypothalamic pituitary function, usually of growth hormone (GH) response to PB and one or more other GH-releasing stimuli. Typically, these have been acute studies using relatively low doses of PB, which offer the opportunity to investigate not only the pituitary responses to, but also the adverse effects of, small doses of PB in humans.

Insight into the acute hormonal effects of PB is available from an abundant literature that describes its use as a clinical test of pituitary GH reserve. Normally, the synthesis and secretion of growth hormone are regulated by two hypothalamic peptides: GH-releasing hormone (GHRH), which has a stimulating role, and somatostatin, which has an inhibitory role. In normal humans, GHRH and its analogues stimulate GH secretion in a dose-dependent fashion (Giustina et al., 1990; Cordido et al., 1993, 1995; Penalva et al., 1993; Arvat et al., 1995, 1997a,b), but its release is substantially modulated by cholinergic neurotrans-

mission (Ross et al., 1987; Ghigo et al., 1990a,b; Giustina et al., 1991; Bellone et al., 1992). Anticholinergic drugs abolish the GH response to certain physiological and pharmacological stimuli (Massara et al., 1986; Casanueva et al., 1990), while PB, an AChE inhibitor, stimulates GH secretion when administered alone (Ghigo et al., 1990b) and enhances the GH response when administered with GHRH or arginine (Giustina et al., 1990).

Mediated by a cholinergically provoked decrease in the hypothalamic release of somatostatin, PB substantially augments the dose–response relationship of GHRH to GH secretion in normal subjects and patients with a variety of pathological disorders (Giustina et al., 1990; Ghigo et al., 1990a, 1996a,b). These physiological effects of PB have been studied extensively in normal volunteers, and the drug has been widely used to test the hypothalamic–pituitary responses, particularly those of GH, of patients with many different disorders. In fact, combining GHRH with PB or arginine—substances that inhibit hypothalamic somatostatin release—is the most powerful means of testing the secretory capacity of human pituitary somatotrope cells (the GH response is always greater than 19 μg/L), which accounts for its wide clinical use (Ghigo et al., 1996b). Accordingly, there is considerable information about the acute administration of PB given as a diagnostic test of hypothalamic–pituitary function in normal volunteers and patients.

As a diagnostic test, PB is generally administered as a single 30–180-mg dose, usually with GHRH, which sometimes produces acute transient symptoms. Among several thousand patients, reported in numerous studies (e.g., Ross et al., 1987; Ghigo et al., 1990a,b,c, 1996a,b; Giustina et al., 1990, 1991; Murialdo et al., 1991,1993; Bellone et al., 1992; O'Keane et al., 1992, 1994; Cordido et al., 1995; Yang et al., 1995; Arvat et al., 1997a,b; Coiro et al., 1998) to whom PB was administered as a diagnostic test, the most common symptoms were muscarinic; abdominal or muscular symptoms usually appeared within 1 or 2 hours of ingesting PB and typically lasted 1–2 hours. Symptoms were monitored for several hours in most studies, and none reported long-term follow-up of symptoms. Abdominal symptoms were described as cramps, increased digestive sounds (due to the movement of gas in the intestines), pain, diarrhea, and nausea, and the principal nicotinic cholinergic symptoms were skeletal muscle and tongue fasciculations (sometimes accompanied by dysarthria).

No central nervous symptoms were reported following PB ingestion, which is particularly noteworthy since it was given to patients with mania (Dinan et al., 1994), depression (O'Keane et al., 1992; Thakore and Dinan, 1995; Coiro et al., 1998), dementia (Murialdo et al., 1991, 1993), schizophrenia (O'Keane et al., 1994), and alcoholism (Coiro and Vescovi, 1997).

A clear dose–response effect on symptoms was not apparent from a review of these studies, although none of the studies were designed to test this effect. However, there was a trend toward greater reporting of symptoms at higher PB dose ranges among subjects given several different doses in the same study. This point is particularly well documented in the results of a study by Yang and colleagues (1995) in which side effects were graded from 1 (mild) to 3 (severe): a

score of 1 in 17 percent (1/6) of subjects given 60 mg of PB; a score of 1 or 2 in 83 percent (5/6) of subjects given 120 mg of PB; and a score of 2 or 3 in 100 percent (6/6) of subjects given 180 mg of PB.

There was general agreement among authors of studies reviewed on several issues: PB is safe and effective when used as a diagnostic test to augment GH responses to GHRH; the test is perhaps the best means of identifying GH deficiency in adults and children; and PB is safe to use in patients with a wide variety of physical and mental disorders. Symptoms were always noted to be brief and tolerable, without requiring medical intervention, and by implication did not require long-term follow-up.

Thus, the main strength of these studies is the careful documentation of acute hormonal responses to PB in normal volunteers of all ages, including children, and in a variety of disease states. Although there is no evidence that PB causes long-term problems when used as a diagnostic test, this point has not been studied. The available studies uniformly show a relatively mild side-effect profile for PB; however, a major weakness is that none were specifically designed to test for adverse effects of PB and many simply ignored this aspect of the drug. Moreover, all of the observations were done over several hours, although several studies administered PB to normal volunteers or patients daily for 2 to 3 days. Nonetheless it is clear that patients with a variety of disease states—many causing severe stress such as thyrotoxicosis or Cushing's disease—had relatively mild acute and transient side effects from PB, and no study reported major clinical problems or obvious acute CNS symptoms after PB administration.

Clinical Studies of PB and Myasthenia Gravis

Myasthenia gravis is an autoimmune disorder characterized by antibody blockade of the ACh receptor of the neuromuscular junction. This immune disturbance results in impaired transmission across the neuromuscular junction and fluctuating weakness in patients with the disease. Myasthenia gravis can affect individuals of all ages with variable and unpredictable severity.

Patients with mild cases may exhibit symptoms of blurred or double vision, while patients with more severe cases may exhibit generalized paralysis and respiratory failure. Some patients with myasthenia gravis also have been shown to have subtle changes in cognitive function related to ACh antibodies binding to ACh receptors in the central nervous system (Iwasaki et al., 1990). These changes may be reversed by treatments for myasthenia gravis, including plasmapheresis (Iwasaki et al., 1990), or with anticholinesterases (e.g., physostigmine) that easily cross the blood–brain barrier and enhance ACh activity (Tucker et al., 1998). Thus, the possible reversal of cognitive dysfunction with anticholinesterases in myasthenics may be an argument against adverse effects of PB on cognition in normal individuals unless there is a difference in the CNS effects of PB in these two populations.

PB (Mestinon) is a prostigmine analogue that has been used since the 1950s to control the myasthenic phenomenon without any major side effects reported (Schwarz, 1956). The usual oral daily dose prescribed to control muscle weakness in myasthenic patients ranges from 120 to 600 mg (Aquilonius et al., 1983), although oral doses may vary from 60 to 1,500 mg per day (Breyer-Pfaff et al., 1990). Despite a short half-life, the pre-dose plasma concentration is relatively stable (Aquilonius et al., 1983). Blood levels of PB do not correlate with the degree of clinical toxicity observed, although they are somewhat predictive of cholinergic crisis in myasthenia gravis patients (Breyer-Pfaff et al., 1990).

In a large series of myasthenic patients followed for 5 years, 34 percent of those receiving PB had one or more, mostly mild, side effects (Beekman et al., 1997). The most common effects were gastrointestinal (30 percent); infrequent effects were hypersalivation (6 percent), increased perspiration (4 percent), urinary urgency (3 percent), increased bronchial secretion (2 percent), rash (1 percent), and blurred vision (1 percent). Only 1 percent of the patients had to stop the drug because of stomach complaints.

A number of studies dating back to the 1950s consistently have shown PB to be safe and effective in the treatment of myasthenia gravis (Schwab and Timberlake, 1954; Schwab et al., 1957; Osserman et al., 1958). PB provides short-term benefit and is still used in the treatment of myasthenia gravis, despite the introduction of surgery, immunotherapy, and intravenous gamma globulin as therapeutic modalities.

Oral doses of 180 mg per day of PB are also used to treat the generalized fatigue and pain of patients with postpolio syndrome. Even though the drug is ineffective in improving the symptoms associated with postpolio syndrome, patients tolerate the medication well with minimal side effects (Trojan and Cashman, 1995; Trojan et al., 1999, described later in chapter).

In conclusion, the majority of studies in the clinical literature focus on the efficacy of PB in the treatment of myasthenia gravis; however, most of the studies reviewed were not designed to determine adverse health effects. The widespread use of this compound has not typically been associated with short- or long-term adverse effects in myasthenia gravis patients.

Clinical Studies of PB in Veterans and General Health Outcomes

Information on symptoms and health status of 41,650 soldiers (6.5 percent of whom were women) who received PB at the onset of combat during Operation Desert Storm has been described (Keeler et al., 1991). Thirty medical officers in close daily contact with the combat units they served were queried retrospectively about the general physiological response of soldiers to PB and potential adverse effects. The reported effects represent impressions of the unit officers and were based on the number of clinic visits, discontinuations of PB, hospitalizations, and evacuations attributed to PB that came to their attention. Based on these anecdotal reports, the authors concluded that soldiers taking PB under combat conditions performed at full effectiveness, but experienced more

minor gastrointestinal and urinary symptoms than expected. An estimated 1 percent of the soldiers had effects from PB for which they sought medical advice, and less than 0.1 percent had effects that warranted its discontinuation.

On their return from the Gulf War, two women sought counseling about their pregnancies because they realized they had been exposed, during their first trimester, to PB and anthrax vaccine (Sarno et al., 1991). PB has been used in pregnancy without producing fetal anomalies, but Sarno and colleagues (1991) note that there are no controlled studies of PB and reproductive risks in females.

Keeler and colleagues (1991) suggest that the higher proportion of personnel experiencing adverse physiological effects than reported in peacetime evaluations may result from the combined effects of anticipated combat, sleep deprivation, and life in the field. Due to the limitations of the retrospective and uncontrolled nature of the data, Neish and Carter (1991) challenged these conclusions.

Acute poisoning with PB is uncommon, but Almog and colleagues (1991) report on nine cases of self-poisoning in men ($n = 6$) and women ($n = 3$) age 17–19 years associated with misuse of PB that was widely distributed as a prophylactic drug during the Gulf War. The doses ranged from 390 to 900 mg, but no CNS toxicity was observed in the nine patients. Mild to moderate cholinergic symptoms developed within several minutes after ingestion and lasted up to 24 hours. Two of the nine patients presented with muscarinic signs such as abdominal cramps, diarrhea, nausea, hypersalivation, vomiting, and urinary incontinence. One patient was observed to have transient nicotinic effects of fasciculations and weakness. The authors concluded that PB intoxication is self-limited and that PB is well tolerated by young adults (Almog et al., 1991). Thus, the various studies of PB exposure provide evidence that PB is not highly toxic, even at high doses or when taken during combat conditions.

Clinical Studies of PB and Neuromuscular Effects

A group of 17 patients with postpoliomyelitis syndrome (PPS) and 10 controls were studied for response to ChE inhibitors (Trojan et al., 1993). Patients with PPS suffer generalized weakness as well as signs and symptoms of muscle weakness thought to be due to neuromuscular junction transmitter defects. Patients responsive to the short-acting anticholinesterase edrophonium were subsequently treated with oral PB at 180 mg daily in divided doses for a period of a month. Side effects included intestinal cramps, diarrhea, muscle cramps, anxiety, blurred vision, and increase in urinary frequency. Using mobility and subjective fatigue as end points, clinical response to PB was measured before treatment and 1 month after treatment. Nine of the seventeen patients reported improvement in fatigue, including reduction in systemic fatigue as well as less muscle fatigability. One patient experienced reversible worsening of symptoms of fatigue. The nine responding patients continued the drug for a mean of 1.2 years despite associated mild gastrointestinal side effects. No other significant side effects were observed.

In a case series of six elderly patients (ages 65–73) suffering from frequent "drop attacks," Braham (1994) reported on the efficacy of PB at 60 mg twice a day. None of the patients presented with any other cardiovascular or neurological diagnosis to explain these sudden falling episodes, and most benefited without suffering side effects from long-term therapy (the longest treatment period was 4 years at time of the report).

Thus, it appears that PB seems to be well tolerated and without significant neuromuscular side effects at the prescribed dose. However, as noted above, the number of patients observed for long periods with sensitive measures of motor function is insufficient to determine whether or not there is a long-term or latent effect of PB on the neuromuscular circuit.

Clinical Studies of PB and Behavioral or Cognitive Function

Molloy and Cape (1989) investigated the effects of PB on cognitive function in 15 elderly patients with Alzheimer's disease. Their rationale for the study was the fact these patients have widespread dysfunction of central cholinergic systems necessary for memory and other higher functions. In addition, they noted that a number of previous studies with physostigmine had shown an improvement in the cognition of Alzheimer's disease patients after treatment. Using a randomized, double-blind, crossover study design, seven elderly men and nine women (mean age 76 years) were treated with 240 mg of PB in divided doses over a 26-hour period. No significant difference in cognitive testing was found between subjects treated with PB and placebo. No side effects were observed. The authors suggested the possibility that doses were too low or the treatment period too short for observation of cognitive or untoward effects.

As noted earlier, patients with myasthenia gravis demonstrate subtle changes in cognitive function (Iwasaki et al., 1990) that are reversed with treatment, including plasmapheresis or anticholinesterases that cross the BBB (e.g., physostigmine) (Tucker et al., 1998).

Thus, these studies offer little evidence for long-term cognitive effects of PB in normal populations. The study of elderly patients with Alzheimer's disease treated with 240 mg over 1 day showed no difference in cognitive testing between placebo and treatment subjects; no side effects were noted. However, Alzheimer's patients would not necessarily be expected to reliably report untoward effects. Further, the possible reversal of cognitive dysfunction with anticholinesterases in myasthenics and the lack of evidence that the CNS of myasthenics responds differently to PB than that of the nonmyasthenic population might argue against adverse effects of PB on cognition in normal individuals.

Clinical Studies of PB and Cardiovascular-Related Effects

Because AChE inhibitors have been used for more than 50 years in the treatment of myasthenia gravis, clinical records may provide evidence of ad-

verse effects in this group of patients. From a pool of more than 1,000 patients with myasthenia gravis treated with ChE inhibitors, Arsura and colleagues (1987) present detailed clinical descriptions of drug-related hypotensive events in 12 patients (7 men, 5 women, mean age 62.6 years). Among these 12 case studies, 8 hypotensive events occurred after edrophonium, 2 after neostigmine, and 2 after PB. The proximal causes of documented syncopal or near-syncopal episodes were severe sinus bradycardia, junctional bradycardia, or complete atrioventricular (A-V) dissociation in nine patients and paradoxic sinus tachycardia in two others. None had obvious preceding signs of cholinergic excess, but all 12 patients had documented new exposure to or upward dose adjustment of their AChE-inhibiting medications, temporally consistent with the onset of the hypotensive episode. Also, the patients showed a strong tendency to respond to atropine and/or to reduce or discontinue use of the anticholinesterase drug, arguing against myasthenia gravis as the primary cause of the adverse event. Based on this review of more than 1,000 patients with myasthenia gravis treated with AChE inhibitors, the estimated frequency of such drug-related syncopal or presyncopal events is approximately 1 percent. The authors advise extreme caution in the use of this class of medications in all patients with pre-existing conduction defects and in the elderly, the groups that seem most prone to medication-precipitated hypotensive episodes.

Considerable clinical experience of adverse cardiovascular events also exists in relation to the use of AChE inhibitors for postanesthesia reversal of nondepolarizing muscle relaxants (e.g., d-tubocurare and pancuronium). Owens and colleagues (1978) studied 93 elderly patients (age > 65 years) undergoing general anesthesia for elective surgery. In this setting, 43 patients were treated with neostigmine and atropine and 50 with PB and atropine (all agents administered intravenously), and monitored for 90 minutes for the occurrence of postoperative cardiac dysrhythmias. Twenty-three percent of all patients experienced abnormal cardiac rhythms. Among those treated with neostigmine, 35 percent exhibited arrhythmias compared to 14 percent among the PB group, a statistically significant difference. The most commonly observed dysrhythmias were due to prolonged atrioventricular conduction resulting in bradycardia or A-V block. The authors concluded that the muscarinic effects of PB are associated with fewer cardiac side effects than those of neostigmine.

Arad and colleagues (1992a) explored the cardiovascular effects of PB on eight hypertensive patients treated with beta-adrenergic blockers (propranolol and atenolol). In this double-blind, crossover study, patients were treated with 30 mg of oral PB or placebo three times daily for 2 days. PB caused no significant effect on heart rate, plasma catecholamine levels, or resting blood pressure. Both systolic and diastolic blood pressure increased with exercise intensity, although a small but statistically significant decrease in diastolic blood pressure with exercise was noted during PB treatment. The authors attributed this to a mild decrease in peripheral vascular resistance (PVR) induced by the parasympathomimetic action of PB. Given the fact that patients with essential hypertension demonstrate increased PVR and that beta-blockers tend to further increase

PVR, this effect might be a beneficial one. No adverse reactions were observed. The investigators concluded that these results indicate the relative safety of the combination of PB and beta-blockers. However they acknowledged that such a small sample and brief period of treatment with PB do not rule out possible rare side effects, especially among people with significant cardiac conduction defects or congestive heart failure.

Teichman and colleagues (1985) described a beneficial interaction between PB and the ventricular antiarrhythmic agent disopyramide. Use of disopyramide is limited by its anticholinergic side effects (xerostomia, dryness of nose and eyes, urinary retention, constipation, abdominal pain, and blurred vision). To prevent or relieve these side effects, a sustained-release form of PB was administered to 27 of 106 disopyramide-treated patients referred for arrhythmia therapy. Doses varied from 90 mg every 12 hours (the usual dose) to as high as 180 mg every 8 hours. When PB was administered prophylactically, none of the patients receiving disopyramide developed anticholinergic side effects compared with 29 percent of those not treated with PB. Of the 10 patients treated at the onset of anticholinergic symptoms, 7 had complete resolution of their symptoms and 3 improved. There were no cardiac side effects attributable to PB, nor was there any evidence of decreased efficacy of disopyramide among PB-treated patients. The authors concluded that since PB caused no measurable decrease in disopyramide blood levels, the prevention or amelioration of anticholinergic side effects was related to its cholinomimetic activities and PB might be a useful agent for the treatment of disopyramide-related anticholinergic side effects.

In conclusion, studies of PB in patients with underlying medical problems are difficult to generalize to the normal healthy population because the disease state may affect the outcome of the response. The greatest experience with the cardiovascular side effects of PB is drawn from the clinical histories of patients with myasthenia gravis, usually at doses higher than those likely to have been used in the Gulf War. The incidence of drug-related cardiac arrhythmias appears to be approximately 1 percent and reversible with a decrease in dosage of PB. It is unlikely that the arrhythmias were due primarily to the underlying illness since myocardial involvement in myasthenia gravis is relatively uncommon. Older patients and those with pre-existing conduction abnormalities are at highest risk. It is of interest that studies of other patient groups also support the relationship between greater age and the risk of untoward cardiac events. This concordance among patient groups supports the association observed between age and cardiovascular side effects in PB-exposed individuals in the general population.

Other drug interactions have been described (see Box 6.1). In the literature reviewed above, no clinically significant side effects were noted when patients on beta-adrenergic blocking agents were treated with PB; although the study was brief and the number of subjects relatively small, no cardiac side effects of PB were observed.

BOX 6.1
Drug Interactions

- Beta-adrenergic blockers (beta blockers): PB might interact with non-selective beta-blockers, potentially precipitating bronchospasm.
- Alpha-adrenergic agonists: Epinephrine and norepinephrine act additively to increase PB lethality in mice when given prior to PB.
- Other cardiac drugs: PB may interact with calcium channel antagonists or other direct-acting vasodilators which might intensify orthostatic hypotension (IOM 1996, Health consequences).
- Quinidine: PB might accentuate atrioventricular block, resulting in hypotension.
- Anti-malarials drugs (e.g., quinine, quinidine, and chloroquine): Might potentiate gastrointestinal effects including diarrhea associated with PB.
- Quinolone antibiotics have been associated with diffuse weakness and dyspnea in patients with myasthenia gravis treated with PB.
- Acetazolamide: may cause exacerbations of muscle weakness in patients with myasthenia gravis, an interaction which might be of concern given the potential use of acetazolamide by troops to prevent altitude-related symptoms.
- Hormones: Oral contraceptive pills and corticosteroids may decrease plasma cholinesterase levels by up to 50 percent. This effect might be of importance in PB-treated patients also treated with succinylcholine and ester local anesthetics which depend upon plasma cholinesterase for metabolism.
- Thiopental (Pentothal): Because thiopental can induce bronchospasm and reduce blood pressure, careful monitoring of troops with asthma or hemorrhage (hypovolemia) if previously treated with PB has been suggested.
- Opioids might interact with PB to enhance bradycardia. The vasodilatory effect of morphine could also augment any hypotensive effects of PB.
- Neuromuscular blocking agents: Non-depolarizing (curariform) agents such as Pavulon, which compete for acetylcholine, could be antagonized by pre-treatment with PB. Depolarizing agents such as succinylcholine, which cause tonic stimulation of nicotinic receptors, would be augmented by pre-treatment with PB.
- Local anesthetics: PB could interact with the ester or procaine-like compounds such as cocaine and tetracaine by inhibiting plasma cholinesterase which is necessary for their metabolism. Such inhibition could result in high serum concentrations of these drugs, potentially leading to cardiac arrhythmias or central nervous system toxicity.

SOURCE: Madsen (1998).

Clinical Studies of PB and Respiratory Effects

The respiratory effects of PB have been studied in clinical investigations of normal subjects, asthmatics, and individuals with myasthenia gravis. All of the

investigations of respiratory effects have been short term. The opposing effects of PB administration in myasthenics complicate respiratory studies in this population. For example, Ringqvist and Ringqvist (1971) studied respiratory effects of intravenous or intramuscular PB in 10 moderate to severe myasthenics aged 17–64 years. They found an increase in airway resistance (R_e) and in maximum inspiratory ($P_E$max) or expiratory pressure ($P_i$max) in all subjects within 60 minutes of drug administration, but also observed an increase in vital capacity (VC). All of the subjects reported subjective improvement in respiration with PB administration. Pulmonary function parameters rapidly returned to normal with administration of a sympathomimetic drug.

In another study of 21 myasthenics, Shale and coworkers (1983) observed a decrease in airflow (FEV%) and an increase in airway resistance at 90 to 120 minutes following a dose of 60 or 120 mg PB given orally. The effect was completely blocked when ipratropium (a muscarinic blocker) was given simultaneously with PB. In a post hoc analysis, the increased airway resistance was found to be present only in subjects with airflow obstruction present at baseline.

De Troyer and Borenstein (1980) administered PB to myasthenics and normal controls and followed pulmonary function for 1–2 hours after drug administration. The control subjects ($n = 4$) were males aged 29–37 years. Following a 2-mg intramuscular dose there was no change in static lung volumes, conductance, or flow–volume curves. The PB dose was not titrated and there was no measurement of ChE level following administration.

A clinical study of 12 normal and 13 asthmatic subjects by Ram and colleagues (1991) measured pulmonary function for 24 hours after PB administration. The subjects were all male nonsmokers. Normal and asthmatic subjects received 60 and 30 mg of oral PB, respectively. The mean decrease in ChE activity was 28.2 percent in normals and 23.3 percent in asthmatics. A small decrease in FEV_1,[6] but not FEV% or PEF (peak expiratory flow), was observed following PB at rest and postexertion in the normal subjects. The decrease correlated with ChE depression and was statistically, but not clinically, significant. Among the asthmatic subjects there was an exercise-induced increase in airway resistance, but no effect of PB at rest or with exercise.

In summary, the literature on respiratory effects of PB is sparse and inconsistent. Taken together, the existing studies suggest that mild increases in airflow obstruction may occur within 1–2 hours of PB administration, but the effects are subclinical and rapidly reversible. Asthmatics may experience a small increase in exercise-induced airway resistance following PB administration.

Healthy-Volunteer Studies

A number of studies were conducted in healthy military and nonmilitary volunteers to evaluate the tolerance of prophylactic doses of PB that might be

[6]FEV_1 (forced expiratory volume) is the volume of gas expelled from the lungs during the first second of expiration after maximal inspiration.

used in combat to protect troops in the event of exposure to chemical warfare agents. These studies were reviewed for reports of general health outcomes and adverse effects on different organ systems.

Neuromuscular Studies in Healthy Volunteers

In a double-blind, placebo-controlled crossover study, Graham and Cook (1984) evaluated the effects of PB on 24 healthy male volunteers between the ages of 21 and 35, measuring a wide variety of performance measures including neuromuscular strength by grip testing and perceived level of exertion. Thirty milligrams of oral PB or placebo was administered three times daily for 5 days. No significant difference in strength or perceived exertion between PB and placebo-treated subjects was observed. The PB regimen produced the expected mean level of inhibition of plasma ChE, although large variation in inhibition between individuals was observed. No evidence of adverse health effects was found.

In another double-blind, placebo-controlled study of 35 healthy volunteers, Glikson and colleagues (1991) measured the effects of oral dosing of PB on direct tests of muscle strength and endurance. Subjects received either PB as 30-mg tablets or a placebo every 8 hours for 10 days. Before and during treatment, four subjects in the PB group and two in the placebo group also underwent electrodiagnostic studies (e.g., electromyography [EMG], nerve conduction velocity, and repetitive strength testing) of neuromuscular function. Subjects (16 placebo and 19 PB treated) underwent baseline tests of these parameters and were retested after 8 days of treatment and again 5 days after the cessation of treatment. Blood AChE levels showed a mean decrement of 23 percent, compared to baseline, among PB-treated subjects during therapy. Posttherapy AChE levels were identical to baseline.

Test parameters were analyzed as the percentage change from baseline for every variable in each subject. Measurements of handgrip strength as well as isokinetic elbow flexor and extensor strength did not differ between the placebo and PB groups. Electrodiagnostic studies of subjects treated with PB showed no significant change from baseline during or after the treatment. Some direct, standardized measurements of muscle strength (knee flexor and isokinetic strength) showed a small but statistically significant improvement on day 8 among placebo- but not PB-treated subjects. Conversely, knee extensor endurance showed a slight but statistically significant decrease on day 8 in the placebo group, whereas this variable remained unchanged in PB-treated subjects. Posttreatment strength measurements were not significantly different from baseline in either group.

The authors attributed the differences in some measures of muscle strength and endurance to large fluctuations in performance among the placebo group. It is possible that the improvement in knee flexor and extensor muscle strength at day 8 among placebo-treated subjects reflected only a training effect that was prevented by PB in the treatment group. However this would not explain the return to baseline 5 days posttreatment. The slight but statistically significant

advantage in day 8 muscle endurance for PB-treated subjects also was not observed posttreatment, giving additional credibility to the authors' conclusions and arguing against the clinical significance of both of these findings. The authors' overall conclusion is that treatment of healthy subjects with PB at oral daily doses of 90 mg for 8 days caused no significant neuromuscular effect.

In summary, two studies of healthy volunteers treated with PB, at doses and for a time comparable to those for some military personnel during the Gulf War, found no evidence of significant, clinical neuromuscular abnormality. One study (Graham and Cook, 1984) of men between the ages of 21 and 35 tested grip strength and perceived exertion, and found no significant PB-related changes.

The second study (Glikson et al., 1991) included more detailed strength testing as well as electrodiagnostic studies, but was limited by the extreme youth of its subjects (18–20-year-old males). Any age-related propensity to develop the symptoms seen in Gulf War veterans, which has been reported, would not be apparent in this study. The follow-up period for both studies was very brief—only 1 to 2 weeks after the initiation of therapy—making it difficult to determine whether abnormalities might occur at a later time. It should also be noted that one set of parameters, the electrodiagnostic tests, were performed on only four treated subjects, limiting the ability to rule out the potential for EMG, nerve conduction velocity, and repetitive strength testing abnormalities, which might have been detected by studying a larger, more diverse group for a longer period of time.

Neurobehavioral and Cognitive Function in Healthy Volunteers

A number of controlled studies have shown subtle neurobehavioral changes in subjects exposed to low doses of PB. Cognitive tests such as visuomotor coordination, dynamic visual acuity, reaction time, digit symbol, critical flicker fusion, and mood have been used to assess the effect of PB on performance. Individual performance on these tests was not significantly affected, although when the data were pooled, visual–motor coordination decreased (Borland et al., 1985). One study showed that in addition, perceptual speed and reaction time were impaired by heat and exercise rather than by PB (Arad et al., 1992b). Four subjects exposed to military doses of PB did not show compromised visual performance when tested for low-contrast acuity with dim illumination, a demanding task used to assess aviators' visual ability (Wiley et al., 1992). Flight performance was not impaired by four doses of 30 mg PB in subjects tested on the A-4 simulator (Izraeli et al., 1990).

A 1984 study of psychomotor performance by Graham and Cook (1984) evaluated the effect of PB on multiple parameters of performance including psychophysiological indices, psychomotor performance, cognitive function, and other central processing functions. In addition, multiple task performance measurements were included in order to assess whether the drug had any impact on conditions of increased workload, where a subject was required to perform two tasks simultaneously (dual-task performance). Finally, subjective measures of symptoms including fatigue, perceived workload, and depression were applied.

Twenty-four healthy men between the ages of 21 and 35 participated in this placebo-controlled, double-blind, crossover study. An oral dose of 30 mg PB or a placebo was administered three times daily for 5 days with crossover after a 1-week period of "washout." Testing of study variables was performed on days 2, 4, and 5 of treatment and 3 days after cessation of each treatment.

On day 2 of PB intake, performance was worse on a visual probability monitoring task (a test of perception and reaction time). On days 4 and 5, PB treatment was associated with decrements in dual-task performance. For example, when a visual tracking task was performed simultaneously with a memory search task, there was a tendency for the memory task to be more disrupted under PB treatment than under placebo conditions. Interestingly, PB significantly improved tests of hand steadiness on days 4 and 5. No adverse health effects were observed, and measures of subjective state and daily work activities failed to distinguish a difference between PB and placebo treatment. The authors concluded that PB, in the doses used, is well tolerated by healthy young men. However, the subtle decrements in cognitive function were considered noteworthy since they related to complex functions, which might be of particular importance in military operations. The rapid and precise actions required of an F-16 pilot were cited as an example of such performance demands (Graham and Cook, 1984).

Thomas and colleagues (1990) studied the effects of PB on cognitive performance of ten U.S. Navy divers during extended heat and warm water exposures. After prolonged pre-dive heat exposure, followed by a 3-hour dive, tests of short-term memory, learning acquisition, vision, and coordination were administered. For 2 days prior to and during one of the test exposures, subjects were treated with oral PB. Before and during another exposure, placebo was orally administered. The dose of PB is not stated. Short-term memory and measures of learning were impaired after heat exposure dives. No differences between PB and placebo-treated subjects were observed for any of the study parameters. The authors interpreted the findings as revealing that heat stress has a clear effect on the performance of complex cognitive tasks, but that PB had no such effect on behavioral or psychophysiological performance when administered either alone or in combination with heat stress (Thomas et al., 1990).

In summary, the 1984 study by Graham and Cook (1984) of the effects of PB on multiple parameters of performance provides a remarkably comprehensive and subtle assessment of psychomotor and cognitive functions in volunteers treated with doses of PB likely to have been used in the Gulf War. Although tests of many psychomotor and cognitive functions are unaffected by PB, there appears to be a trend for decrements in performance of complex tasks involving rapid shifts of attention. The study suggests subtle effects of this drug on cognition, reaction time, and complex performance that are not, however, supported by the prevailing concepts regarding the inability of PB to penetrate the blood–brain barrier. Although there was no evidence of persistent effects 3 days after discontinuation of the drug, suggesting that these are short-term effects, no long-term follow-up was reported.

The study of navy divers (Thomas et al., 1990) under heat stress conditions showed no effect of PB on neurobehavioral parameters. Unfortunately, the dose of PB is not stated, making these results difficult to interpret. Again, no long-term follow-up was reported. Thus, these studies offer little evidence with which to predict possible long-term effects of PB on normal patients.

Cardiovascular-Related Studies in Healthy Volunteers

It has been suggested that acute or chronic low-dose exposure to anticholinesterase compounds may cause subtle, subclinical effects that might be masked by tolerance or by CNS compensatory mechanisms. Izraeli and colleagues (1991) proposed that pharmacological challenge with a cholinomimetic agent (atropine) could prove useful in unmasking the latent muscarinic effects of AChE inhibitors such as PB.

To this end, eight healthy male subjects (mean age 29) were enrolled in a placebo-controlled, single-blind crossover study in which a 30-mg oral dose of PB or a placebo was administered every 8 hours for a total of four doses. Dosing began 24 hours before the commencement of noninvasive cardiopulmonary monitoring, with the fourth and final dose given 75 minutes before the monitoring session. After baseline recording of electrocardiogram (ECG), respiratory rate, and cardiac power spectra, these parameters were measured for 7 minutes after each of nine consecutive increasing intravenous doses of atropine. Volunteers reported no symptoms; no changes in mean heart rate or heart rate power spectrum were noted after PB treatment alone. The usual bimodal effect of atropine on heart rate (low doses ordinarily cause bradycardia, whereas higher doses cause tachycardia) occurred, but PB at the higher doses of atropine blunted this expected effect. The expected inverse effect on the respiratory peak (mediated mainly by parasympathetic input) was also observed, with the respiratory peak increased at the low dose of atropine and decreasing incrementally at higher atropine doses. Moreover, analogous to the findings for heart rate, this expected atropinic effect was attenuated by pretreatment with PB. These results indicate that PB-mediated cardiac rate effects are "unmasked" by interaction with atropine and that such effects are present even at asymptomatic dose levels of PB.

Another study of healthy volunteers by Nobrega and colleagues (1996) was designed to explore the effects of PB on cardiac cholinergic responses. Eight healthy volunteers (five men and three women, mean age 27) participated in a randomized, double-blind crossover trial comparing cholinergic effects of PB as a single 30-mg oral dose to placebo. Each subject underwent a 12-lead EKG, and three noninvasive cardiovascular maneuvers (respiratory sinus arrhythmia, Valsalva maneuver, and 4-second exercise test) were performed before and 2 hours after taking PB or placebo. PB was found to have a negative chronotropic cardiac effect (i.e., slowing of the heart rate) as evidenced by increased R-R intervals[7] at rest and during the three autonomic tests. However, the drug had no

[7]R–R interval is the time interval between two consecutive ventricular depolarizations.

effect on compensatory reflex changes in heart rate as determined by the magnitude of the ratio of the longest to the shortest R–R intervals with each of the maneuvers. The authors concluded that despite tonic, dynamic reflex responses remain intact, suggesting a possible cardioprotective role for PB on post-myocardial infarction patients.

Harriman and colleagues (1990) designed a study to assess the possibility of PB treatment-related decrements in psychophysiological performance. Their preliminary publication dealt with the measurement of physiological parameters, including cardiovascular effects. A double-blind placebo-controlled crossover study of 24 male, trained A-10 pilots (mean age = 29) was carried out in flight simulators (with 12 pilots wearing chemical defense garments) after administration of either placebo or the standard PB regimen (30 mg every 8 hours) for 3 days. The pilots were first screened by measurement of plasma ChE inhibition after 30 mg of oral PB. Although there was marked individual variation of levels, in none of the pilots was ChE inhibition greater than 40 percent. Respiratory rate was not affected by PB. Heart rate and heart rate ratio (beats per minute/respirations per minute) were both decreased by PB. PB treatment also led to reports of 27 symptoms among 12 (50 percent) of the pilots. In contrast, placebo treatment resulted in only five (20 percent) pilots reporting six symptoms. The most common symptoms among PB-treated subjects were gastrointestinal upset, fatigue, confusion or giddiness, and headache. The authors concluded that the standard chemical warfare pretreatment regimen is safe for personnel who are prescreened for PB sensitivity.

In summary, in one small study (Izraeli et al., 1991), using relatively low doses (30 mg every 8 hours) of PB and causing no symptoms in healthy volunteers, subtle changes in heart rate and its normal respiratory fluctuations were observed. Another small study (Nobrega et al., 1996) of healthy subjects revealed slight negative cardiac chronotropic effects shortly after a single 30-mg oral dose of PB. However, this finding did not seem to be clinically significant because it was asymptomatic and autonomic reflex mechanisms easily compensated for the mild slowing in heart rate. The study of mission-ready pilots (Harriman et al., 1990) treated with the standard chemical warfare pretreatment regimen demonstrated gastrointestinal upset, fatigue, confusion or giddiness, headache, and decreases in heart rate and in the ratio of heart rate to respiratory rate without clinically significant cardiovascular symptoms.

These studies in healthy volunteers are limited because of the small number of participants and the brief duration of therapy without follow-up. It is, therefore, difficult to determine the significance of the findings or to compare their results to individuals with chronic illnesses or with genetically determined variations in cholinesterase activity.

Respiratory Studies in Healthy Volunteers

A study of FEV_1 was done on six, healthy male volunteers with no history of asthma following intravenous (i.v.) administration of PB (0.143 mg/kg,

maximum 10 mg) and atropine (0.0143 mg/ kg, maximum 1 mg) (Feldt-Rasmussen et al., 1985). A maximum decrease of 27 $\pm$ 5 percent in ChE was observed 5 minutes after injection, but no decrease in FEV_1 was observed during 90 minutes of follow-up. The study demonstrated that the muscarinic effects of PB are completely blocked by atropine.

Gouge and colleagues (1994) reported on pulmonary function changes following PB administration among soldiers during the Gulf War. Ten asthmatics and six healthy normal soldiers received 30 mg PB orally and had a vital capacity measurement and lung exam every 2 hours for 8 hours. No consistent change in VC was observed, but seven of the ten asthmatics reported increased chest tightness 2–6 hours after PB. No respiratory symptoms were reported by the normal controls or by any of the 264 other members of the unit who received a 30-mg PB dose. There was no measurement of airflow obstruction in this clinical study, and the reported symptoms cannot be interpreted. The study was not blinded or controlled (with a placebo), symptoms may have been due to other etiologies (e.g., stress), and may not have been correlated with airflow obstruction.

In summary, there are few studies in normal subjects on the respiratory effects of PB. Respiratory function is of interest because the muscarinic effects of PB overdosage include increased bronchial secretions and possibly smooth muscle contraction, which can increase airflow obstruction. Nicotinic side effects include muscular weakness, which can also impair respiratory function. However, as noted above, the existing clinical studies suggest that mild increases in airflow obstruction may occur within 1–2 hours of PB administration, but the effects are subclinical and rapidly reversible.

Thermoregulation in Healthy Volunteers

Seidman and Epstein (1989) published a review of the existing literature on the thermoregulatory effects of anticholinesterase agents in 1989 (the earliest of the six reviewed studies). Case studies of acute organophosphate poisoning in humans are noteworthy for the frequent finding of severe hyperthermia, which often occurred late (more than 24 hours after initial presentation) and lasted for several days in patients surviving the initial toxicities (reviewed in Seidman and Epstein, 1989). Less commonly, OP-poisoned individuals presented with hypothermia, probably due to a combination of effects including hypothalamic dysregulation, excessive sweating, and muscle paralysis leading to obliteration of the shivering response.

In an investigation of heat stress and PB effects on thermoregulatory indices, eight heat-acclimated healthy young men were subjected to repeated bouts of exercise in a hot–humid environment after receiving four oral doses of either PB or placebo in a double-blind crossover fashion (Seidman and Epstein, 1989). No significant effects of PB could be demonstrated on physiological parameters, including final rectal temperature, amount of heat stored in the body, dry heat exchange, sweat excretion, and sweat efficiency.

Other studies of small numbers of healthy male volunteers of comparable design have yielded similar results. In one such study (Epstein et al., 1990a), a slight but statistically significant decrease in heart rate was observed but was asymptomatic. In this study the mean age of volunteers was 23.5 years and the eight subjects were preacclimatized.

Epstein and colleagues (1990b) also studied heat exercise performance in eight subjects (mean age 23.5 years) under the added stress of wearing chemical protective clothing. Using a study design similar to those previously described, the volunteers were subjected to 170 minutes of exercise heat stress in protective clothing 4 hours after the fourth 30-mg oral dose of PB. Heart rate, heat storage, and sweat rates were similar in PB- and placebo-treated subjects. Nonevaporative (dry) heat exchange was significantly greater for PB-treated subjects than for controls. The authors concluded that heat stress in subjects wearing chemical warfare (CW) protective garments could lead to severe increases in body heat after 2 hours, but pretreatment with PB did not further decrease exercise performance beyond the limitations presented by heat, exercise, and CW protective clothing.

Wenger and colleagues (1993) studied thermoregulatory effects of dry heat, exercise, and PB treatment in a 7-day, double-blind, crossover study. Seven subjects (mean age 22) received 30 mg PB or placebo every 8 hours and exercised on a treadmill in a dry heat environment. PB increased sweating and evaporative water loss and lowered skin temperature during exercise compared to placebo. PB had no significant effect on rectal (core) temperature, oxygen uptake, or fluid balance. Although PB alone had no significant effect on heart rate, there was an interaction between the day of study and PB treatment on heart rate such that heart rate changed from a decrease of 0.4 beats per minute on day 1 to a decrease of 7.9 beats per minute on day 4. Similar temporal interactions occurred with regard to lowering of skin temperature with exercise. An interaction between increased sweating and treatment day also occurred such that the effect of PB changed from an increase of 0.1 liter of sweat on day 1 to 0.3 liter on day 7. The authors concluded, however, that standard CW prophylactic doses of PB had no clinically significant effect on thermoregulatory response in subjects exposed to exercise and dry heat.

Two studies of PB on thermoregulation during cold exposure were reviewed. Prusaczyk and Sawka (1991) studied the effects of a single 30-mg dose of PB on thermoregulatory responses in six men (mean age 21.8 years) subjected to cold water immersion for up to 180 minutes 2 hours after ingestion of PB or placebo. Cold exposure increased metabolic rate, ventilatory volume, and respiratory rate similarly in PB- and placebo-treated subjects. PB had no significant effects on rectal temperature, mean body temperature, subjective thermal sensations, plasma cortisol levels, or plasma volume. However, severe, but transient, abdominal discomfort caused termination of cold exposure in three of six PB experiments. Investigators concluded that PB did not increase susceptibility to hypothermia but could result in severe abdominal cramping that might limit cold tolerance.

Roberts and colleagues (1994) studied thermoregulatory responses in subjects exposed to exercise and cold air. Seven healthy volunteers (mean age 20)

participated in a 14-day, double-blind, placebo-controlled, crossover study with 7 days of PB treatment (30 mg three times a day and 7 days of placebo), during which exercise and cold testing were performed on days 2 and 3 and again on days 6 and 7. PB and control treatments resulted in similar metabolic rates, body temperatures, and regional heat concentrations. No differences were noted between earlier and later measurements with regard to any of the thermoregulatory and metabolic parameters. It was concluded that the study showed no "acute or chronic" effects of PB treatment on thermoregulation and metabolism during exercise in cold air.

In summary, review of studies of the effects of PB on healthy male volunteers are in general agreement with the conclusion that PB (at doses similar to those taken by troops during the Gulf War) results in no clinically significant perturbation of thermoregulatory homeostasis.

It should be noted, however, that some physiological parameters in some of the studies showed statistically significant differences for PB treatment compared to placebo. These PB-related findings include the following: mild, asymptomatic decrease in heart rate among heat- and exercise-stressed subjects (Epstein et al., 1990a); increase in nonevaporative heat exchange during heat stress with CW-protective clothing (Epstein et al., 1990b); increased sweating, increased evaporative water loss, and lowered skin temperature with dry heat stress and exertion, with an increased change in exercise-induced sweating and drop in skin temperature and heart rate on later days of treatment (Wenger et al., 1993); and episodes of self-limited but severe abdominal cramping in cold-exposed subjects (Prusaczyk and Sawka, 1991). These findings are of unknown clinical significance, but because of the rapid return to baseline, these PB-associated changes are not likely to be harbingers of long-term effects.

It is also important to notice that of the five studies (36 subjects) in which average weight and body surface area are stated,[8] the measurements are similar to those expected for an average healthy male (the standard "70-kg man"). Smaller men and most women are substantially different from these subjects with regard to weight and body surface area (e.g., an average fit young American woman weighs approximately 55–65 kg and has a body surface area of about 1.5–1.75 m^2). Since CW preexposure PB dosages are fixed (30 mg every 8 hours), the experimental findings in these studies could underestimate drug effects on many personnel.

Finally, these studies are limited by the small numbers of subjects and high degree of fitness among participants. Further, none of the studies were chronic in duration, nor did any study report long-term follow-up of experimental subjects. Hence, delayed effects of PB on thermoregulation, although unlikely, require further study.

[8]The mean weight and body surface area for the volunteers are 72.8 kg and 1.81 m^2, respectively (means weighted according to the number of subjects in each study).

Ocular or Visual Effects in Healthy Volunteers

Graham and Cook (1984) studied the effects of PB on multiple psychomotor parameters (see earlier discussions of neuromuscular effects and behavioral and cognitive function) as well as visual performance in 24 healthy male volunteers. In a double-blind, placebo-controlled, crossover study comparing 5 days of treatment with 30 mg of oral PB or placebo three times daily, investigators tested a number of visual function parameters, including spatial resolution by contrast sensitivity; neural transit time by steady-state visual evoked response; visual acuity by Snellen eye chart; and depth perception by biopter test. Testing was performed on days 4 and 5 of treatment and again 3 days after cessation of treatment. Interestingly, on days 4 and 5, subjects receiving PB had significant improvement in tests of depth perception compared to subjects on placebo. No significant drug-related effect on stationary visual acuity, contrast sensitivity, or stereopsis was noted. The drug regimen produced the expected level of inhibition of plasma ChE. However large differences between individuals in inhibition (range = –21.7 to +8.3 percent) were observed. Regression analyses were performed to determine the relationship between individual differences in ChE inhibition and study variables. These analyses indicated that as the level of enzyme inhibition increased, performance on tests of visual acuity decreased while depth perception improved.

Borland and colleagues (1985) investigated the effect of 3 days of oral PB at 30 mg every 8 hours on visual function and visual–motor coordination. Four healthy men between the ages of 19 and 27 participated in this double-blind, placebo-controlled, crossover study. After PB treatment, the mean critical flicker fusion threshold was significantly raised, an improvement in performance compared to that of subjects on placebo. PB-treated subjects performed dynamic visual acuity tasks with fewer missed responses than those on placebo. Visual–motor coordination was impaired with PB. No effect of PB was seen on pupillary diameter, static visual acuity, macular threshold, or kinetic perimetry. The authors suggest that the mild but statistically significant improvement in some visual performance tests was due to an increase in the level of arousal caused by increased stimulation of CNS cholinoceptive sites. The mild decrease in visual–motor coordination was attributed to either the peripheral anticholinesterase activity of the drug or a direct nicotinic action on the neuromuscular junction.

Also using a placebo-controlled, double-blind, crossover design, Kay and Morrison (1988) studied the effects on vision of a single 60-mg oral dose of PB in 14 male volunteers 18–40 years of age. In the first of a two-part experiment, contrast sensitivity to stationary oscilloscope-generated gratings showed a small but significant increase of 7 percent, which was attributed to a small reduction in pupillary diameter. Secondly, contrast sensitivity to laser interference fringes was tested, and with this method, which bypasses the optic media, no effect of PB was found. The authors concluded that PB could be used as a pretreatment for chemical warfare agents without a deleterious effect on stationary visual function.

Wiley and colleagues (1992) investigated the effects of PB on a variety of measures of visual performance. Four healthy male pilots-in-training (mean age 23) were treated with 30 mg oral PB every 8 hours for 3 days. On the day prior to beginning treatment (day 1), subjects underwent measurements of spatial resolution ability, dark adaptation, refractive error, and oculomotor function. Testing was repeated on each of 3 days of treatment (days 2, 3, and 4) and then again on day 5 during which no drug was administered. Mean contrast sensitivity thresholds showed no significant change during PB treatment. Six tests of oculomotor function were performed, and PB did not affect four of these tests. Fusional divergence, however, did show an effect of PB with test distance (6 m versus 70 cm), a finding the authors thought could not be due to PB (although no alternative explanation was offered). Pupillary diameter and refractive error both demonstrated significant PB-associated changes with relative miosis and myopia, respectively. The authors concluded that at the study dosage, PB would not adversely affect an aviator's visual ability. The investigators do acknowledge that longer periods of treatment might have increased effects on vision.

Alhalel and colleagues (1995) studied the use of 30 mg PB three times a day as an agent to prevent the ocular anticholinergic effects of double-dose transdermal hyoscine patches. The hyoscine (scopolamine) patch has been commonly used against motion sickness, but its use is limited by the anticholinergic symptoms of impairment of near vision and accommodation as well as mydriasis. Investigators studied 47 healthy men (ages 18 to 21) in a placebo-controlled double-blind fashion. Subjects were treated with either double-strength or placebo hyoscine patches and PB tablets or placebo. Treatment duration was 2 days. PB significantly ameliorated hyoscine-induced impairment of near vision and accommodation but did not prevent the mydriatic effect of the patch. The authors concluded that PB at this dose was an effective antagonist of some ocular anticholinergic effects (impaired near vision and accommodation) of the hyoscine anti-motion sickness patch.

In summary, studies of the effects of PB on ocular or visual function in healthy volunteers reveal at least mild effects on visual function, however, the specific effects, their mechanism of action, and their significance are unclear.

No severe PB-related changes in stationary visual acuity were observed in most studies, although some findings are suggestive of such an effect. Graham and Cook (1984) noted that visual acuity decreased as the percentage of plasma AChE inhibition increased, while an opposite relationship occurred with depth perception, which improved as AChE inhibition was decreased by PB treatment. Therefore, PB treatment seemed to augment depth perception while having a negative effect on visual acuity. The latter effect might be due to drug-related induction of mild myopia (nearsightedness), which was indirectly observed by Wiley and colleagues (1992), who found that PB prevented the hyperopia (farsightedness) caused by the hyoscine motion sickness patch.

With the usual CW pre-exposure dose and schedule, some studies found miosis (i.e., decrease in pupillary diameter) (Wiley et al., 1992). PB might also

improve accommodation as evidenced by its prevention of hyoscine-induced impairment of accommodation (Alhalel et al., 1995).

Borland et al. (1985) described PB-induced improvement in the threshold for the fusion of flickering light and augmentation of dynamic visual acuity, findings the authors attributed to stimulation of CNS cholinergic actions. If these findings were related to CNS action, it would argue against the more generally accepted idea that PB cannot significantly penetrate the blood–brain barrier (see earlier discussion). These findings are important for the issue of possible long-term PB effects but currently are preliminary and unconfirmed. Other authors conclude that any observed effects on visual function are due to the peripheral action of PB on the pupillary sphincter, ciliary muscle, or oculomotor muscles. In general, the visual function effects of PB at routine CW pre-exposure doses are mild and reversible. However, the possibility of long-term or latent effects is difficult to assess since the studies reviewed were all of short duration.

Gastrointestinal Effects in Healthy Volunteers

Oigaard (1975) examined and compared the effects of PB and metoclopramide on upper gastrointestinal activity in 40 normal volunteers and 8 postoperative laparotomy patients. Using simultaneous recordings of electrical action potentials and intraluminal pressure, investigators compared the effects of the two drugs on upper gastrointestinal activity in healthy and surgical subjects. Measurements were taken after an oral dose of 40 mg of metoclopramide or an intravenous dose of 1.5 mg of PB. Both drugs had significant excitatory effects, with metoclopramide being the stronger stimulant of gut activity (possibly because the experimental dose of PB was relatively less potent than that chosen for metoclopramide).

A 1967 report of pharmacologic regulation of salivary gland secretion assessed PB's effects on the saliva flow rate (Mandel et al., 1967). Both neostigmine and PB increased salivary flow, PB to a lesser degree than neostigmine.

In conclusion, studies of PB administration to normal volunteers demonstrate its expected short-term stimulatory effects on gastrointestinal motility and salivation, without any noted adverse effects.

Overall Performance in Healthy Volunteers

A number of studies have been carried out to assess overall measurements of both physiological and behavioral or cognitive parameters in healthy subjects treated with PB.

Izraeli and colleagues (1990) examined the effect of four doses of PB (30 mg every 8 hours) on flight skills in a placebo-controlled, double-blind, crossover study. The technical performance of 10 healthy male pilots (21–33 years) was tested in a flight simulator. No decrement in flight performance was observed among subjects treated with PB compared to placebo. Although they were mild in quality, slightly more symptoms were noted in PB-treated subjects.

A similarly designed study by Gawron and colleagues (1990) of 21 healthy male pilots found no significant decrements in performance in a flight simulator aircraft after standard treatment with PB. Pilots were unable to subjectively determine whether they received PB or placebo. However, another study of similar design (Brooks et al., 1992) of 24 A-10 pilots performing complex maneuvers in a flight simulator did not reveal a significant difference except in regard to one task. In a simulation of low-level penetration into a target area, pilots on PB were "killed" by surface-to-air missiles (SAMs) 25 percent more frequently; this finding was statistically significant. The authors attributed this effect to the "simulation factor" without explaining why simulation would preferentially exert nonpharmacological effects on PB-treated subjects.

Other investigators addressed the issue of PB effects on performance in combination with other physiological stressors. Forster and colleagues (1994) studied the effects of gravitational acceleration (G-force) stress and the usual CW pre-exposure PB regimen on overall performance in five healthy male volunteers (average age 26). This placebo-controlled, double-blind study assessed physiological and cognitive parameters. No statistically significant difference between PB- and placebo-treated subjects was noted for pulmonary function, heart rate, QT intervals,[9] PR intervals,[10] handgrip strength, or tolerance for acceleration. Cognitive performance was not systematically statistically affected by PB. Likewise, a similarly designed study of 10 Navy divers examining exercise, immersion, and warm temperature stresses (Doubt et al., 1991), showed no drug-related changes in heart rate, minute respiration, oxygen consumption, tidal volume, handgrip strength, or perceived exertion. Another study of like design measured the effects of standard doses of PB combined with heat–exercise stress and wearing CW protective clothing on eight healthy male volunteers (Arad et al., 1992b). PB had no significant effect on cognitive and physiological parameters but a statistically significant increase was found in "shortness of breath" ($p < .005$) in the PB-treated group. The authors concluded that in light of the absence of objective changes in respiratory function, this symptom is not likely to be of clinical significance. Cook and colleagues (1992) studied the simultaneous effects of heat, exercise, and standard PB doses for 7 days on seven healthy male subjects (mean age 21.4 years). PB was associated with a 4-mm decrease in resting diastolic blood pressure, a 0.050-mm decrease in pupillary diameter, a 3 percent decrease in handgrip strength, and a 0.1°C higher final rectal temperature. Although statistically significant, effects of this magnitude are unlikely to be clinically important. Subjects were unable to distinguish between days on placebo and days on PB.

In summary, the preponderance of evidence in studies reviewed above supports minimal effects of PB on overall motor, physiological, and cognitive functioning when normal healthy subjects are tested with complex tasks or physical

[9]QT is the time interval that reflects the duration of ventricular depolarization and repolarization.

[10]PR is the time interval between atrial and ventricular depolarizations.

stressors. However, this statement must be understood to carry some important caveats:

- Most subjects were extremely young males (<30 years) in exemplary physical condition (flight-ready pilots, Navy divers, medically screened volunteers). Whether the mild and/or equivocal performance effects (such as possible decrease in ability of pilots to avoid SAM attack in one simulation study) or the slight physiological changes or symptoms noted in others are clinically important in older or less well-screened individuals remains to be determined.
- Long-term studies are unavailable at this time. In the longest of the above-cited investigations (Cook et al., 1992), a trend for decrease in hand strength became significant only after the full 7 days of PB treatment. The possibility exists that this change represents a downregulation of the ACh receptor after excessive stimulation by PB block of AChE. Long-term follow-up is needed to determine the possibility of a chronic adverse neuromuscular effect.
- The number of subjects in each study is small and may not identify a sensitive subpopulation of individuals who might respond with greater adverse effects due to genetic variability (e.g., variants of BuChE activity).

Genetic and Population Pharmacokinetics and Pharmacodynamic Studies

Clinical Research Services, Inc. (1996), studied the safety, tolerance, and pharmacokinetics or pharmacodynamics (PK/PD) of PB in 45 male and 45 female healthy volunteer subjects in a randomized, placebo-controlled study. Subjects were divided into six groups by gender and weight (low, medium, and high weights for both sexes), with 15 subjects in each group. Within each group, 10 subjects received 30 mg of PB three times daily for 21 days and 5 subjects received placebo on the same schedule and for the same duration. Side effects were mild and similar between PB- and placebo-treated subjects except for mild gastrointestinal muscarinic symptoms that occurred with greater frequency among the PB-treated group. Blood chemistry, hematology, hormone levels, and electrocardiography showed no significant changes between those receiving PB and those on placebo. Plasma concentration of PB showed gender and weight effects early in the study, and maximum concentration of PB was significantly associated with weight (lower weights associated with higher serum concentrations).

Marino and colleagues (1998) describe a 1998 population PK/PD analysis of PB: 60 healthy men and women between 18 and 45 years of age received either 30 mg PB or placebo orally every 8 hours for 21 days (30 PB subjects and 30 placebo subjects). The PK model that best fit plasma PB levels was a two-compartment open model with first-order absorption and first-order elimination from the central compartment. The PD model that best fit RBC AChE activity was an inhibitory E_{max} model with an effect compartment linked to the central compartment. The results of this study show that the pharmacokinetics of PB are both gender and weight dependent. Due to wide individual variations, 30 percent

of the population may not achieve CW protective red blood cell AChE inhibition levels (>10 percent inhibition) on this standard regimen.

Loewenstein-Lichtenstein and colleagues (1995) presented a case study of an Israeli soldier who suffered from severe symptoms of nausea, insomnia, weight loss, fatigue, and depression while receiving standard doses of PB during the Gulf War as CW pre-exposure prophylaxis. The symptoms resolved some weeks after PB was discontinued. His past medical history was noteworthy for an earlier episode of postanesthesia apnea (succinylcholine used as paralytic agent). The authors analyzed the soldier's BuChE activity spectrophotometrically and also delineated his (and his family's) genotype by recombinant expression in *Xenopus* oocytes. These studies documented the subject to be homozygous for the most common variant allele of BuChE, with enzyme serum activity about one-third that associated with the usual genotype. The authors noted that homozygous carriers of this particular allele comprise about 0.04 percent of people of European ancestry but may be as high as 0.6 percent in certain subsets of this population. They posited that such atypical homozygotes and possibly even heterozygotes could be at risk for severe symptoms from PB due to the relative deficiency of PB-scavenging effective BuChE. They further speculated that anticholinesterase exposure might lead to long-term adverse consequences with symptoms that are not incompatible with those of Gulf War veterans, and that combined exposures to other ChE inhibitors might increase the risk of such outcomes.

In summary, individuals within a healthy population differ widely in their rates of absorption, distribution, elimination (PK), and enzymatic inhibition (PD) related to PB. PK is affected by weight and gender, whereas PD may vary considerably between normal individuals. Genotypic variations of BuChE may cause adverse (conceivably, long term) effects in some populations. Further studies to identify the relationship between such variants and the risk of both acute and chronic health outcomes is warranted (see Chapter 8).

Epidemiologic Studies

A number of clinical and human volunteer studies have investigated a range of potential adverse responses associated with PB exposure. These are described above and essentially indicate minimal toxicity of PB with no irreversible side effects.

Although there have been several descriptive epidemiologic studies of Gulf War veterans, these investigations sought to characterize the nature and frequency of the illnesses reported by returning soldiers and did not examine the association of PB with these illnesses.

There are no analytic epidemiologic studies of the association of PB and adverse health effects in humans. Such studies would optimally have to include both exposed and nonexposed individuals as well as deployed and nondeployed soldiers to control for the environmental conditions associated with combat. A series of reports published by Haley and colleagues attempt to evaluate illnesses in Gulf War veterans (referred to by the authors as Gulf War syndrome) and

specific exposures associated with this syndrome (see Chapter 2). Because these studies used an incomplete study design (i.e., lacked an unexposed comparison group) and demonstrated other weaknesses, they have been strongly criticized on methodological grounds (Cowan et al., 1996; Gordon et al., 1997; Landrigan, 1997; Gray et al., 1998; Wolfe et al., 1998). This section reviews the Haley studies and other epidemiologic investigations of the association between Gulf War veterans' symptoms and PB exposure.

In an initial survey designed to search for syndromes characteristic of Gulf War veterans, Haley et al. (1997b) studied the questionnaire responses of 249 men (41 percent of 606 males from a reserve naval mobile construction battalion [i.e., Seabees]) living in five southeastern states. All respondents had been called to active duty during the Gulf War, and there were no survey responses from nondeployed personnel. Characteristics of the participants (n = 249) and nonparticipants[11] (n = 357) of the 24th Reserve Naval Mobile Construction Battalion (RNMCB-24) indicate that members of the battalion were, on average, older than most deployed forces, with a mean age of 41 years for participants and 37 years for nonparticipants. Participants and nonparticipants were similar in race or ethnicity, education, active reserve status, wartime military rank, and wartime job ranking (Haley et al., 1997b). However, large differences between participants and nonparticipants, respectively, were noted for percentage reporting serious health problems since the war (70 percent versus 43 percent) and percentage unemployed at the time of the survey (11 percent versus 3 percent). Of 249 individuals who responded to the survey several years after deployment, 145 (58 percent) had retired from the military, and the rest were still serving in the battalion. Symptoms included in the survey were those commonly associated with post–Gulf War illness in clinical examinations performed by teams of DoD and Department of Veterans Affairs physicians. From survey responses, the authors used factor analysis[12] to identify six clinical syndromes: (1) impaired cognition, (2) confusion–ataxia, (3) arthromyoneuropathy, (4) phobia–apraxia, (5) fever–adenopathy, and (6) weakness–incontinence.

Psychological testing indicated that veterans with any of the six syndromes had the same psychological profile, which differed only in clinical severity but did not represent posttraumatic stress disorder. Those with syndromes 2 (confusion–ataxia) and 4 (phobia–apraxia) had increased self-reported occupational disability compared to the others. The low participation rate (41 percent) of veterans in the battalion suggests that results may have been affected by selection bias, in that

[11]This term refers to members of the battalion who were contacted by investigators but chose not to participate. Information about nonparticipants was obtained from the wartime roster and from a telephone survey of randomly selected nonparticipants.

[12]Factor analysis is a mathematical–statistical technique used to define the primary dimensions from batteries of test items. Factor analysis aggregates survey responses into statistical groupings of factors that may or may not have biological plausibility or clinical relevance. It is recognized that factor analysis has the potential to generate syndromes that may not be reproduced when a new population is examined.

participants were older, had more illnesses, and were more likely to be unemployed than nonparticipants. The authors believe that such biases were avoided because participants were demographically representative of the entire battalion and because retired veterans were included in the study. Nevertheless, nonparticipants were less likely to report having had a serious illness since the war and were more likely to be employed. Moreover, the average age of this group of Seabees was greater than that of most active duty units, suggesting that study subjects might not accurately reflect the nature of illnesses in other military units.

At least 25 percent of ill veterans in the battalion studied had symptoms that the authors believe suggested generalized neurological injuries, mainly combinations of damage to the brain or brain stem (e.g., cognitive and vestibular dysfunction), the spinal cord and peripheral nervous system (e.g., paresthesias of the extremities, muscle pain and weakness, joint pain, urinary incontinence), and the autonomic nervous system (e.g., chronic diarrhea).

There is concern that the survey sample used by Haley and colleagues was small, increasing the potential to generate spurious results (Gray et al., 1998). Another potential source of bias is the numerous medical examinations and media contacts of study subjects before the survey was conducted and the reliance on self-reports of symptoms and adverse responses to PB that occurred many years earlier (Gray et al., 1998). The study population was a reserve naval command, whose members were often employed full-time in nonmilitary careers, with occupational exposures and subsequent confounding health risks, and thus may not be representative of the general population of Gulf War veterans.

The most important of the Haley reports with regard to an association with PB exposure is described as "a cross-sectional epidemiologic study" (Haley and Kurt, 1997). This study of the association between self-reported wartime exposures and self-reported symptoms in a small proportion (41 percent) of the 606 members from the RNMCB-24 relies heavily on the syndromes developed by factor analysis of symptoms reported by these same veterans (Haley et al., 1997b). The survey instrument used by Haley and colleagues to elicit self-reported exposures and symptoms in participating members of the battalion was developed by Haley and colleagues and pretested on five Gulf War veterans. After revision, the survey instrument (exposure and symptom booklet) was again pretested on five additional Gulf War veterans. It is important to note that associations reported by Haley and Kurt (1997) are based on comparisons of responses by ill and non-ill Gulf War veterans and do not include comparisons of responses from nondeployed veterans.

The authors report that the prevalence of syndrome 1 (impaired cognition) was greater among veterans who reported wearing flea collars during the war (5 of 25, 20 percent) than in those who never wore them (7 of 229, 3 percent; RR [relative risk] 9.7 [3.0–24.7], $p < .001$). Syndrome 1 was not associated with subjects having taken PB or reporting adverse effects from PB.

The prevalence of syndrome 2 (confusion–ataxia) was eight times greater among veterans who reported having experienced a likely CW attack. The prevalence of syndrome 2 was not higher in people who reported having taken a

larger number of PB tablets but was higher in soldiers who recalled having experienced more severe adverse effects from PB (χ^2 for trend, $p < .001$). Syndrome 2 was more prevalent among veterans who believed they had been involved in a chemical weapons exposure (18 of 108, 17 percent) than those who did not believe they were so involved (3 of 141, 2 percent; RR 7.8 [2.3–25.9], $p < .001$) and was higher in veterans who reported having been in a sector of far northeastern Saudi Arabia (a site allegedly exposed to CW agents) on the fourth day of the air war (6 of 21, 29 percent) than those who did not report having been there (15 of 228, 7 percent; RR 4.3 [1.9–10.0] $p < .004$). Effects of perceived chemical weapons exposure and perceived advanced adverse effects from PB were synergistic using a Rothman S test.

The study found that the prevalence of syndrome 3 (arthomyoneuropathy") increased with the frequency of reporting certain adverse effects for PB and reporting the use of large amounts of government-issued insect repellent containing DEET ($p < .001$ for both).[13] Each of the three syndromes was associated with a different set of risk factors. Haley and Kurt conclude that the risk factor associations observed in their study suggest that these three syndromes may represent variants of OPIDN due to varying degrees of exposure to organophosphate nerve agents potentiated by interactions with other chemical exposures and older age.

A major limitation of the design of this cross-sectional study, and its subsequent interpretation, is the lack of comparable symptom and exposure data for a nondeployed or non–Gulf-deployed military population. Based on discussions with the committee, Dr. Haley is aware of this limitation and plans to pursue studies of comparable phenomena in nondeployed military populations.

A further study by Haley et al. (1997a) evaluated neurological function in 23 Gulf War veterans with symptoms (cases) and 20 Gulf War controls (10 deployed and 10 not deployed, from the same battalion) in a nested case-control study. This study found impairment in ill Gulf War veterans compared to controls on each of two global measurements of brain dysfunction (the Hallstead Impairment Index, $p = .01$, and the General Neuropsychological Deficit Scale, $p = .05$. There were significant differences on 20 of 89 tests with endpoints that did not depend on volitional action by subjects (e.g., evoked potentials); cases were more impaired on 18 tests and controls on 2 tests ($p <0.001$). By contrast, there were significant differences on 15 of 76 tests with end points depending on volitional action; cases were more impaired on 9 and controls on 6 ($p = .30$).

Cases with impaired cognition (syndrome 1) were more impaired on brainstem auditory evoked potentials; those with confusion–ataxia (syndrome 2) were more impaired on the Halstead Impairment Index, asymmetry of saccadic velocity, and somatosensory evoked potentials; and those with arthromyoneuropathy (syndrome 3) were more impaired on caloric stimulation than the controls. An independent examination by six neurologists blinded to the case-

[13]Note: p-value for trend reported is <0.001 in abstract and .0001 in Table 3, p. 234 (Haley and Kurt, 1997).

control status of the subjects indicated that about two-thirds of all veterans (cases and controls) had at least one abnormal neurological finding (most frequently reduced strength of the lower extremities); however, there were no significant differences in the frequency of neurological findings in the cases and controls. The examining neurologists and study investigators reviewed the findings on each subject individually and concluded that the clinical and laboratory findings were nonspecific and not sufficient to diagnose any known neurologic syndrome in any subgroup of subjects (Haley et al, 1997a). Nevertheless, the authors expressed the view that the neuropsychological abnormalities seen in Gulf War veterans are likely the result of neurotoxic exposures associated with service in the Gulf War.

This study also has come under criticism. For example, despite the assertion of neurological damage in the 23 most symptomatic subjects, there were no statistically significant differences on neurological examination between cases and controls, and a panel of neurologists concluded that the confusion–ataxia syndrome was not specifically defined by their standard clinical neurological exams. Furthermore, the changes noted on evoked potentials and saccadic velocity were nonspecific compared to larger control populations. Moreover, evoked potentials have limited utility in evaluating patients with possible neurological disorders; their major purpose is to look for evidence of demyelination, which would not be useful in persons suspected of having OPIDN—classically, a distal axonal neuropathy, not a permanently demyelinating disorder of the brain stem and spinal cord. Although a statistically significant difference in interpeak latencies related to PB was reported on the somatosensory evoked potentials in the 23 cases compared with the 20 healthy controls, there were no significant differences between the results in veterans and the authors' own laboratory's established normative values from healthy people. Some "statistically significant" differences are expected by chance alone when a large number of tests are performed on a small number of patients, and in this instance, no adjustment was made for "multiple comparisons."

Some findings had uncertain clinical relevance (e.g., asymmetric nystagmus velocities following caloric stimulation in patients complaining of arthralgia, myalgia, and weakness). Electromyography shows abnormalities in individuals with OPIDN, but single-fiber EMG results were normal in the Haley study. Thus, the clinical data presented provide little evidence with which to conclude that these patients had a neuropathic process, and it is difficult to interpret the findings of a battery of neurological tests under these conditions. For example, five veterans with confusion–ataxia and three with arthromyoneuropathy had peripheral neurophysiological tests (nerve conduction studies, electromyography, single-fiber EMG, and quantitative sensory tests) that were normal, whereas most of the veterans with syndrome 3 (symptoms consistent with peripheral neuropathy) had no findings consistent with this peripheral neuropathy, which calls into question the validity of the symptoms as a measure of neurological damage. The principal limitation of this nested case-control study, in

addition to the limitations of the Seabees study population described above, is the lack of clinical validity of measures used to infer neurological damage.

A more recent study (Haley et al., 1999) investigated genetic polymorphisms of PON1 and BuChE in 45 subjects (cases and controls), who had been reported by Haley and colleagues in the previously described case-control study. The authors measured serum activity levels of various allozymes to test the hypothesis that differences in allozyme levels might have put some Gulf War veterans at higher risk of neurological damage from exposure to environmental chemicals that require these enzymes for detoxification. The study found that ill veterans with the neurological symptom complexes described were more likely to have the R allele of the PON1 gene (heterozygous QR or homozygous R) than to be homozygous Q. They found that low activity of the PON1-type Q arylesterase allozyme distinguished ill veterans from controls better than the PON1 genotype alone or the activity levels of the type R arylesterase allozyme, total arylesterase, total paraoxonase, or BuChE. The authors found that a history of advanced acute toxicity after taking PB was also correlated with low PON1-type Q arylesterase activity.

A major criticism of this study is that type Q, the allozyme of PON1 that most efficiently hydrolyzes several organophosphates (sarin, soman, and diazinon), does not hydrolyze PB (unpublished data from their laboratories cited by the authors); thus, the interpretation of lowered levels of this enzyme is uncertain insofar as it relates to PB use in ill Gulf War veterans.

A study of U.K. servicemen who served in the Gulf War (Unwin et al., 1999) addressed PB exposure. This cross-sectional postal survey of Gulf War veterans (n = 4,248), Bosnia conflict veterans (n = 4,250), and those serving during the Gulf War but not deployed there (n = 4,246) found a greater perception of worse physical health in the Gulf War cohort. Gulf War veterans reported all symptoms and disorders more commonly than the comparison cohort. All of the self-reported exposures showed associations with all outcome measures in the three cohorts. Three exposures were reported more frequently by Gulf War veterans than by the other cohorts: exposure to burning oil-well smoke, vaccination against biological warfare agents, and CW protection measures. Very small proportions of the Bosnia and nondeployed Gulf War era veterans reported exposure to PB, 1.9 percent and 5.2 percent, respectively, compared to 81.6 percent of deployed Gulf War veterans. Nevertheless, the association of symptoms with self-reported PB use was not different between the three cohorts with elevated odds ratios observed for the three principal health outcome measures in all three cohorts.

CONCLUSIONS

Acute Effects

A large number of clinical studies report acute transient cholinergic effects in normal volunteers and patients with a wide variety of clinical disorders given PB as a diagnostic test of hypothalamic pituitary function and patients with my-

asthenia gravis treated with the drug for extended periods. As a diagnostic test, PB is generally administered as a single oral 30–180-mg dose, which produces acute transient cholinergic symptoms in a minority of patients and normal volunteers. Within 1 or 2 hours after ingesting PB, about 25 percent of subjects experience abdominal symptoms, and about 10 percent have muscular symptoms that typically last 1–2 hours. The abdominal symptoms are cramps, increased digestive sounds, pain, diarrhea, and nausea, and the principal nicotinic cholinergic symptoms are skeletal muscle and tongue fasciculations sometimes accompanied by dysarthria. The symptoms are characteristically mild, transient, and tolerable, without requiring medical intervention, and are not accompanied by central nervous system symptoms. Although a clear dose–response effect on symptoms was not apparent from a review of the studies summarized in this report, none were designed to demonstrate this effect. There is, however, a trend toward a greater rate of symptoms at higher PB dose ranges among subjects given several different doses in the same study.

PB is used to control muscle weakness in myasthenic patients, and the daily dose of PB usually ranges from 120 to 600 mg. Studies indicate that about 34 percent of those receiving PB have one or more, mostly mild, side effects, usually gastrointestinal, although a few patients experience other cholinergic symptoms such as hypersalivation, increased perspiration, urinary urgency, increased bronchial secretion, and blurred vision. Patients rarely have to stop the drug because of abdominal complaints.

During the Gulf War, acute accidental poisoning with PB in doses ranging from 390 to 900 mg resulted in mild to moderate cholinergic symptoms within several minutes of ingestion, which lasted up to 24 hours. Patients typically developed muscarinic effects such as abdominal cramps, diarrhea, nausea, hypersalivation, vomiting, and urinary incontinence. The effects are self-limited and well tolerated by young adults.

As noted above, the most extensive information available on the acute effects of PB comes from studies of its use for diagnosis of growth hormone deficiency in adults and children and its therapeutic use in the treatment of myasthenia gravis. These studies, of doses higher than those used for prophylaxis during the Gulf War, consistently indicate that PB is safe and effective in clinical applications. Side effects noted are predominantly gastrointestinal and muscular, and are of a short duration with no long-term residual effects.

Results from other human studies of both clinical and healthy volunteer populations, report the same gastrointestinal and muscular side effects, which are transient and characteristically mild. A small number of idiosyncratic reactions are noted.

The committee concludes that there is sufficient evidence of an association between PB and transient acute cholinergic effects in doses normally used in treatment and for diagnostic purposes.

Chronic Effects

No reports of chronic toxicity related to human PB exposure in clinical or military populations are available. The suggestions by Haley and Kurt, 1997 of a unique manifestation of organophosphate-induced delayed polyneuropathy associated with PB exposure alone or in combination with other wartime exposures, in the absence of acute symptoms of organophosphate toxicity, requires further investigation.

Although Haley and colleagues provide evidence that chronic neurological changes are present in a small number of ill Gulf War veterans compared to a small number of well veterans from the same unit, the validity and causal nature of this association are uncertain due to the large potential for selection and information biases in this study population and the lack of a nondeployed comparison group.

In addition, the evidence that some types of chronic neuropsychological changes may be linked to acute responses to administration of PB, also suggested by Haley and Kurt (1997), is limited by the lack of consistency with results from toxicological and clinical studies; uncertainty about the selection, administration, and interpretation of the neuropsychological tests employed; the highly select nature of the small number of Gulf War veterans studied; and the lack of comparable studies in a nondeployed comparison group.

The epidemiologic data do not provide evidence of a link between PB and chronic illness in Gulf War veterans. Most epidemiologic studies of Gulf War veterans focused on whether a unique Gulf War syndrome exists and on defining its characteristics. Only two epidemiologic studies specifically investigated the possible association of PB use and chronic symptoms among Gulf War veterans (Haley and Kurt, 1997; Unwin et al., 1999). The limitations of the small, selected population studied by Haley have been noted. With regard to PB, Haley's factor-derived syndromes were not associated with taking PB or with the dose of PB. Two of the three syndromes showed an association with self-reported symptoms that are consistent with adverse effects of PB. This finding may result from reporting bias for adverse health syndromes and adverse effects of PB, and provides an inadequate basis for concluding that an association exists. The other epidemiologic study was of U.K. servicemen (Unwin et al., 1999), and all exposures studied (PB, diesel or petrochemical fumes, oil fire smoke, viewing dismembered bodies, etc.) showed an association of similar magnitude with adverse symptoms. Recall and reporting bias may also explain this finding. Thus, neither of these two studies provides a basis for determining that a specific association between PB and chronic adverse health effects exists.

The committee concludes that there is inadequate/insufficient evidence to determine whether an association does or does not exist between PB and long-term adverse health effects.

The available evidence is of insufficient quality, consistency, or statistical power to permit a conclusion regarding the presence or absence of an association in humans. This is true particularly when PB exposures occur in combination with other combat exposures.

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CONTENTS

7

Vaccines

The U.S. military implements a comprehensive immunization[1] program that is designed to protect the armed forces against potential disease risks (Takafuji and Russell, 1990). A standard set of vaccinations is required for each military recruit; this set varies slightly by branch of service. Additionally, when troops are assigned to specific duty stations they are given the vaccinations that are targeted to protect them from the risks found in their assigned geographic locale or that are specifically related to their assignment (IOM, 1996).

During the Gulf War, a number of different immunobiologics (e.g., cholera, meningitis, rabies, tetanus, and typhoid vaccines) were sent to protect against potential exposures to biological threats (Committee on Veterans' Affairs, 1998). Concerns prior to the Gulf War regarding Iraq's offensive biological warfare capabilities, led to decisions that available vaccines should be utilized as preventive measures against biological warfare agents. It is estimated that 310,680 doses of the anthrax vaccine licensed by the Food and Drug Administration (FDA) were distributed to the Gulf War theatre and that 150,000 U.S. troops received at least one anthrax vaccination (Christopher et al., 1997; Committee on Veterans' Affairs, 1998).

[1]The committee used the definitions of the Advisory Committee on Immunization Practices (ACIP), which defines "vaccination" as the physical act of administering any vaccine or toxoid and "immunization" as a more inclusive term denoting the process of inducing or providing immunity artificially by administering an immunobiologic. The ACIP states that although the terms are often used interchangeably, they are not synonymous because the administration of an immunobiologic does not automatically equate with the development of adequate immunity (CDC, 1994a).

Approximately 137,850 doses of botulinum toxoid were sent to the Gulf, and it is estimated that 8,000 individuals were vaccinated (Committee on Veterans' Affairs, 1998). However, medical records from the Gulf War contain little or no information about who received vaccines, how frequently vaccines were administered, or the timing of vaccinations relative to the other putative exposures (OSAGWI, 1999). Further, existing record entries show no consistency in recording the type of vaccine (notations include "A-Vax," "Vacc A," "Vacc B," and "B Vaccination"). A report by the Office of the Special Assistant for Gulf War Illnesses (OSAGWI) found that documents from the Gulf War indicate confusion about where, or whether, the vaccinations were to be recorded (OSAGWI, 1999).

Investigations since the war by the United Nations Special Commission (UNSCOM) and the International Atomic Energy Agency have found that Iraq had biological weapons prior to the Gulf War, but no evidence was found of their release. Investigators found that Iraq had produced 200 biological bombs in 1990; 100 were filled with botulinum toxin, 50 with anthrax, and 7 with aflatoxin (Zilinskas, 1997). Additionally, 13 Al Hussein (SCUD) warheads were found to have contained botulinum toxin, 10 warheads contained anthrax, and 2 contained aflatoxin (USAMRIID, 1996).

This chapter discusses several vaccine-related issues that have been of particular concern to Gulf War veterans. The chapter discusses animal and human studies that have been conducted on the safety of the anthrax vaccine and the botulinum toxoid vaccine. Additionally, the issue of multiple vaccinations is addressed. Finally, the chapter provides an overview of the scientific literature regarding squalene, an issue the committee was asked to address.

The committee issued a letter report on the safety of the anthrax vaccine in April 2000 (IOM, 2000). This letter report was issued in response to a congressional conference report (House Report 106-371). The Institute of Medicine (IOM) is currently conducting a separate two-year study on the safety and efficacy of the anthrax vaccine. That study will review some of the unpublished non-peer-reviewed information that was not available to this committee.

ISSUES IN IDENTIFYING ADVERSE EFFECTS

Vaccines are acknowledged to be one of the most effective tools in the prevention of infectious diseases. Dramatic reductions have been seen in the incidence of many diseases including pertussis, polio, rubella, measles, diphtheria, and mumps in the United States, and globally, smallpox has been eradicated (Keusch and Bart, 1998). In general, individuals experience either no adverse effects from a vaccination or mild local effects (e.g., tenderness, soreness) at the injection site. The administration of some vaccines has been determined to be associated with the potential for transient local or systemic adverse health outcomes (e.g., increased risk of fever, local pain, and/or swelling near the injection site) (Keusch and Bart, 1998). More serious reactions are rare (IOM, 1994). This section highlights some of the major issues that must be considered in deter-

mining whether an adverse health outcome is associated with receiving a vaccine. Several Institute of Medicine reports (IOM, 1991, 1994, 1997) have examined the complex issues involved in vaccine safety in greater depth.

Surveillance

Postmarketing surveillance of licensed vaccines in the United States relies on the voluntary reporting of adverse events. In 1990, the Vaccine Adverse Event Reporting System (VAERS) became operational and is overseen jointly by the Centers for Disease Control and Prevention (CDC) and the Food and Drug Administration. VAERS reports are open-ended forms that allow for description of the symptoms, time course, laboratory tests, vaccine(s) received, and treatment provided. While health care providers and manufacturers are obligated to report specific adverse effects to vaccines covered by the National Vaccine Injury Compensation Program, anyone can file a VAERS report. For the 65,720 VAERS reports received between January 1, 1991, and December 31, 1996, the sources of reports were health care providers (47.3 percent), manufacturers (39 percent), parents (2.4 percent), and others or unknown (11.3 percent) (CDC, 1999b). There is no long-term follow-up mechanism for VAERS reports.

VAERS is a passive reporting system in that it relies on incoming reports. Adverse events are therefore likely to be underreported (IOM, 1997). Further, some reports have incomplete medical information, and the same case may be reported by different sources. VAERS data are useful in signaling potential new adverse events but are limited in their usefulness for assessing the rate or causality of adverse events (IOM, 1994). Although the number of doses distributed is usually available, the number of doses administered is not. Further, the extent of underreporting of adverse events is unknown. FDA and CDC are responsible for monitoring VAERS data to detect unusual trends and occurrences of adverse health effects. This monitoring assists FDA and CDC in responding appropriately to adverse events.

Studies of vaccine safety use either active or passive methods of surveillance in assessing the extent of adverse events. *Active surveillance* methods involve direct follow-up by investigators of all individuals in the study. At a minimum, active surveillance seeks to systematically contact all vaccine recipients at prespecified intervals following vaccination. Often, in addition to posing open-ended questions about possible adverse effects, active surveillance asks explicitly about specific symptoms, and sometimes specific physical or laboratory examinations are conducted**.** *Passive surveillance* methods rely on the vaccine recipient to provide information (e.g., self-reports, surveys) or use other information that may indicate adverse outcomes (e.g., days missed from work, number of visits to the clinic following vaccination). Studies on the botulinum toxoid and anthrax vaccines have relied primarily on passive surveillance approaches and have involved only relatively short periods of follow-up**.**

Difficulties in Detecting Adverse Events Due to Vaccinations in Humans

Detecting adverse events associated with vaccination and determining whether the health outcome is a result of the vaccination are complex tasks due to a number of factors (IOM, 1997) including the following:

- *Lack of long-term follow-up.* Many controlled studies are geared toward monitoring immediate reactions to the administration of the vaccine; subjects are often followed for 6 months at the most.
- *Small sample sizes.* Vaccine trials to determine immunogenicity often involve sample sizes of no more than several hundred individuals. Trials of this size are unlikely to detect rare effects since large sample sizes are needed to detect rare occurrences.
- *Multiple vaccinations.* Individuals often receive several vaccines at a time or over a short period, which makes it difficult to identify the culprit vaccine in the event of an adverse effect. Controlled safety trials of vaccine combinations would have to include as many study groups as there are combinations of vaccines under study, plus at least one reference group, and thus would require large sample sizes.
- *Multiple end points.* The large number of symptoms potentially associated with vaccination complicates surveillance because the reporting mechanism must allow for numerous symptom categories in addition to as-yet-unreported symptom types.
- *Lack of symptoms specific to vaccination.* Since there is no unique clinical syndrome or laboratory diagnosis associated with vaccination, it is difficult to differentiate whether symptoms, such as fatigue or seizures, are due to receiving the vaccine or to some unrelated factor coincident with vaccination.
- *Passive reporting systems.* Passive surveillance systems are most useful as a sentinel for identifying rare or previously unrecognized side effects of newly marketed vaccines and for monitoring the safety of individual vaccine lots. However, these systems do not provide information about the rates of reactions to vaccines. As discussed above, VAERS is a passive system that relies on health care providers, those receiving vaccinations, and others to report health outcomes that may be linked to vaccine exposure in the recent or more distant past. Reporting is likely to depend on the gravity of the effect, the time lapsed since exposure, and the diligence in symptom reporting by the patient's health care workers. Thus, underreporting is an inherent issue. Furthermore, supporting information (e.g., laboratory results) to infer causality may be inaccurate or missing.
- *High vaccination rates.* For widely administered vaccines, it is difficult to find a comparable control group that has not received the vaccine. Unvaccinated individuals constitute a small, highly selected group that may differ from those vaccinated in other aspects and, thus, are not generally suitable as a control group. Further, their small number is unlikely to allow for the study of background rates of rare medical events.

- *Restricted population.* The large majority of controlled vaccine trials are geared toward investigating childhood vaccines. Adverse effects in children may not be generalizable to adults.
- *Progress in vaccine technology.* Earlier vaccines against a particular infectious agent that have been subjected to considerable animal and human study may be substantially different from vaccines currently in use against the same infectious agent. Thus, even careful and extensive earlier studies may not be generalizable to current experience.

Difficulties in Detecting Adverse Events Due to Vaccinations in Animals

Focus on Vaccine Efficacy

Most animal studies focus on the efficacy of the vaccine and do not examine adverse effects. Further, adverse effects that produce symptoms, rather than objectively measurable pathology, are difficult or impossible to study in animals (some studies use animal behavior to infer animal symptoms such as fatigue). The lack of data on adverse events in animal studies can indicate that no adverse events occurred, that adverse events were not monitored, or that adverse events were not sufficiently severe to warrant termination of the experiment. Additionally, most animal studies are concerned with monitoring immediate toxicity to the administration of the vaccine. Animals are most often followed for short periods of time (i.e., weeks to months), and the long-term effects of vaccination are not considered.

Possibility of Immune Stimulation

Studies in animals have generally not considered the mechanism responsible for adverse health effects. In some cases, adverse effects of the vaccination could be due to the toxicity of the antigen in the vaccine, the preservatives or contaminants in the vaccine, or the vaccine adjuvant.[2] Adverse effects may also result from the intended goal of immunization (i.e., stimulation of the immune system). Immune stimulation may result in a state of immune enhancement, hypersensitivity, or an immune-mediated pathological response. The pathological immune response may be directed toward the antigens administered in the vaccine or to self-antigens (i.e., autoimmunity). Immune-mediated tissue damage requires an initial exposure to the antigen to sensitize the animal. The symptoms of immune-mediated tissue damage may occur on subsequent exposures.

A discussion of the immunological reactions that can cause disease has been included in a previous IOM (1994) report and is summarized only briefly here. Classically, such immune-mediated pathology is divided into Types I–IV hyper-

[2]An adjuvant is a substance that is used to increase the immune response to specific vaccine components.

sensitivity as proposed by Coombs and Gell (1968). However, the response to any one antigen may involve a combination of types of hypersensitivity, depending on the antigen dose, site of exposure, and duration of antigen stimulation. Type I hypersensitivity is a response to the antigen that occurs within minutes; symptoms range from a mild rash or urticaria to airway obstruction or acute life-threatening anaphylactic shock. In Type II reactions, antibodies combine with a tissue antigen, resulting in complement system activation and damage to the tissue by the inflammatory process. Drug-induced hemolytic anemia is an example of a Type II hypersensitivity reaction. Type III hypersensitivity involves the interaction of circulating antibody and antigen to form immune complexes that deposit on the walls of blood vessels. The resultant fixation of complement and neutrophil recruitment leads to tissue destruction. The pathology of Type III hypersensitivity tends to be seen in the lung, kidney, joints, and brain in animal studies. A localized reaction in the skin can lead to pain, swelling, induration, and edema. Type IV hypersensitivity or delayed-type hypersensitivity is dependent on the stimulation of antigen-specific lymphocytes and recruitment of macrophages by cytokines. The resultant inflammation leads to tissue destruction. Contact dermatitis to poison ivy is an example of a Type IV hypersensitivity reaction. Animal studies have limitations in detecting adverse effects due to Types II through IV hypersensitivity because the time course of such responses may involve months or years to become clinically apparent in an animal, which is beyond the time frame monitored in most animal studies.

Genetic inheritance strongly influences the immune response, both to immunization and to actual infection (Box 7.1), in animals and humans, which explains why immunologically mediated adverse reactions to vaccination are so variable from one animal, or person, to the next.

ANTHRAX VACCINE

Work on a vaccine to provide protection against the zoonotic disease anthrax[3] began with the work of Pasteur and Greenfield who developed heat-attenuated anthrax vaccines in the 1880s (Turnbull, 1991). In the 1930s, Sterne developed a live attenuated spore vaccine, and versions of this vaccine continue to be used effectively to immunize livestock. The primary use of the anthrax vaccine in humans was initially to protect persons working with animal hair or hides, including goat hair mill workers, tannery workers, and veterinarians.

[3]Anthrax occurs most commonly in herbivores who ingest anthrax spores from the soil. Naturally occurring cases of human anthrax are the result of contact with anthrax-infected animals or contaminated animal products. There are three clinical forms of human anthrax infection: inhalation, cutaneous, and gastrointestinal. Inhalation anthrax naturally occurs only rarely, but the mortality rate approaches 100 percent (Fauci et al., 1998). Since 1950, the incidence of the disease in animals and man has dropped markedly due in large part to the availability of the vaccine, the use of antibiotics, and the implementation of strict quarantine laws in many countries (Whitford, 1987).

Box 7.1
Genetics and the Immune Response

Genetic factors can influence the host's response, including the immune system's response, to foreign antigens in many ways—for example, metabolism of the antigen, antigen processing, alteration of self-antigens, stimulation or suppression of the immune response, the nature of the humoral immune response (e.g., different immunoglobulin subclasses), the nature of the cellular immune response (e.g., Th1 versus Th2 response), or the development of an autoimmune response/disease. There are several examples of genetic factors that have been associated with the development and severity of infectious diseases. Polymorphisms (variant gene patterns) for the HLA gene alter the risk for severe pulmonary tuberculosis. Certain polymorphisms of the gene for cytokine tumor necrosis factor are associated with more severe malaria.

The immune response is also modulated by hormonal factors. Sex hormones affect the immune response as do adrenal hormones; estrogen tends to enhance the immune response, and cortisone tends to diminish it. The immune response is also modulated by various cytokines (molecules released by immune system cells that direct actions by other immune system cells), some of which increase and others of which decrease the immune response.

Normally the human body is "immunologically tolerant" to its own self-antigens, and the immune system does not attack the body's own tissues. However, in some circumstances this tolerance appears to be broken, resulting in autoimmune disorders. Theoretically, there are several ways in which autoimmune diseases are thought to occur:

- Environmental agents (such as chemicals) may enter the body and alter certain native body substances (usually proteins) so that these native substances are perceived by the immune system as being foreign.
- Antigens from an infectious agent may have a structure that is similar to that of one or more normal host antigens. When the immune system reacts to the foreign antigen, it may also "inadvertently" attack the normal host antigens that are structurally similar.
- Genetic factors that affect the immune response may encourage an autoimmune attack. For example, autoimmune diseases are often strongly associated with certain histocompatibility antigen (HLA) genes. There is evidence that infection with Coxsackie virus is more likely to cause Type I diabetes mellitus in people with the HLA DR4 allele.
- Estrogens may also affect the clinical expression of certain autoimmune diseases. A number of autoimmune diseases are found more frequently in females including: Hashimoto thyroiditis, Graves disease, systemic lupus erythematosus, and scleroderma. HLA B27 associated spondyloarthropathies are found more frequently in males, although it is not clear that this is caused by male sex hormones.

SOURCES: Ahmed et al., 1999; Albert and Inman, 1999; Cooper et al., 1999; Miller, 1999; Rao and Richardson, 1999.

Currently three anthrax vaccines are commercially available for human use. A live attenuated spore vaccine for humans was developed in the 1940s from a Sterne strain derivative and has been tested and used on a large scale in humans in the countries of the former Soviet Union (Shlyakhov and Rubinstein, 1994a). The British and U.S. anthrax vaccines were developed in the 1950s using filtrates of anthrax strains. Protective antigen, one of the three toxin proteins (discussed below), produced by the anthrax bacillus is the immunogenic component of both the U.S. and the U.K. vaccines. The British vaccine is an alum-precipitated cell-free filtrate of an attenuated Sterne strain culture and was licensed in 1979 (Pile et al., 1998).[4] The U.S. vaccine is an aluminum hydroxide-adsorbed cell-free culture filtrate of an unencapsulated strain (Pile et al., 1998).

The anthrax vaccine was first produced on a large scale in the United States by Merck, Sharp, and Dohme in the 1950s for Fort Detrick (GAO, 1999c). Production was turned over to the Michigan Department of Public Health (MDPH) in the 1960s, and some changes were made in the manufacturing process; a different strain of anthrax was used in the MDPH vaccine, and the yield of protective antigen was increased (GAO, 1999c). In 1966, the Investigational New Drug (IND) application was submitted to the Division of Biologic Standards (DBS), formerly in the National Institutes of Health (NIH). Product licensure for Anthrax Vaccine Adsorbed was granted on November 10, 1970. The safety study of the anthrax vaccine submitted to the DBS contained information on the administration of approximately 16,000 doses. In 1985, an FDA advisory panel reviewing the status of bacterial vaccines and toxoids categorized the anthrax vaccine in Category 1 (safe, effective, and not misbranded) (FDA, 1985).

In December 1997, the Secretary of Defense announced that all U.S. military forces would receive anthrax vaccinations for protection against the threat of biological warfare. The Anthrax Vaccine Immunization Program (AVIP) began vaccinations in March 1998; the first personnel vaccinated were members of units deployed or scheduled to deploy to high-threat areas (Claypool, 1999).

It is estimated that 68,000 doses of the U.S. anthrax vaccine were distributed from 1974 to 1989; 268,000 doses in 1990; and 1.2 million doses from 1991 to July 1999 (Ellenberg, 1999). The exact number of people who received the vaccine is not known. The current dosing schedule is 0.5 ml administered subcutaneously at 0, 2, and 4 weeks and 6, 12, and 18 months, followed by yearly boosters. BioPort Corporation (previously Michigan Biologic Products Institute, formerly MDPH) manufactures the U.S. vaccine, approved for use in men and women age 18 to 65 years. The vaccine contains no more than 2.4 mg aluminum hydroxide per 0.5-ml dose as an adjuvant, formaldehyde as a stabilizer (final concentration ≤ 0.02 percent), and benzethonium chloride (0.0025 percent) as a stabilizer (BioPort, 1999; Friedlander et al., 1999).

[4]During the Gulf War, British troops received the U.K. anthrax vaccine administered simultaneously with the pertussis vaccine in an adjacent site in the deltoid muscle (U.K. Ministry of Defence, 2000).

The length of the dosage schedule, along with questions about the extent of the efficacy of the current vaccine against newly engineered strains of anthrax, has led to ongoing research efforts to produce a second-generation recombinant vaccine (Ibrahim et al., 1999; Nass, 1999). Additionally, researchers hope that new processes will be designed to ensure a more precise amount and a more highly purified component of protective antigen in the vaccine (GAO, 1999b; Russell, 1999).

Toxicology

Anthrax disease results from exposure to the bacterium *Bacillus anthracis* through three primary routes: cutaneous, inhalation, and gastrointestinal. Regardless of the route of exposure, the presence of the organism provokes an immune response. Both humoral and cell-mediated immunity play a role in defending against *B. anthracis* (Turnbull et al., 1986; Shlyakhov and Rubinstein, 1994b). An individual who has recovered from *B. anthracis* infection is protected against a subsequent infection with the same organism. Some studies have correlated protective immunity in animals with the antibody response to *B. anthracis* (Barnard and Friedlander, 1999), but other studies have not confirmed this finding (Little and Knudson, 1986; Turnbull et al., 1986).

Knowledge of the pathogenic mechanisms of *Bacillus anthracis* can provide insight into the potential adverse effects associated with administration of the various anthrax vaccines (Friedlander, 1997; Ibrahim et al., 1999). *B. anthracis* is pathogenic by virtue of its capsule and protein exotoxins. The capsule of the bacillus is encoded by an extrachromosomal plasmid pX02 (Little and Knudson, 1986). Another plasmid (pX01) encodes for all three toxin proteins: edema factor (EF), lethal factor (LF), and protective antigen (PA). PA, the transport protein, is required for transport of the enzymatic proteins EF and LF into the target cells of the host; PA must be present for the toxins to confer virulence (Ibrahim et al., 1999). In vitro studies of the toxins have revealed that PA binds to cells and undergoes limited proteolysis, which exposes a potential binding site for LF and EF. The LF–PA and EF–PA complexes enter the target cell by receptor-mediated endocytosis, followed by translocation of LF or EF to the cytosol (Friedlander, 1986; Leppla et al., 1990). The edema toxin complex, composed of EF and PA, acts through calmodulin-dependent adenylate cyclase activity to cause the excessive fluid accumulation that is associated with anthrax infection (Leppla, 1982; Ibrahim et al., 1999). The lethal toxin complex, composed of LF and PA, is the primary cause of shock and death (Ibrahim et al., 1999). Lethal toxin is a zinc metallopeptidase that is rapidly cytolytic for macrophages in vitro and induces the release of the cytokine tumor necrosis factor (TNF) from macrophages (Hanna et al., 1993). Studies in mice indicate that TNF and interleukin-1, in particular, contribute to the death induced by injection of lethal toxin (Friedlander, 1986).

Mechanism of Action

Live attenuated spore vaccines. Live spore vaccines used in veterinary practice, as well as the Soviet Sterne live spore vaccine used in humans, are (pX01+, pX02–) unencapsulated strains of *B. anthracis.* These vaccines are administered intramuscularly, subcutaneously, or by scarification. The host mounts an immune response to the organism and its toxin proteins. Live spore vaccines induce a humoral immune response. However, the live spore vaccine also elicits a cell-mediated immune response (Shlyakhov and Rubinstein, 1994b). The absence of the capsule reduces the virulence of the organism, yet the bacillus can still produce the toxin proteins PA, EF, and LF. Thus, the formation of active edema toxin and lethal toxin is possible.

Protective antigen vaccines. The U.K. and U.S. vaccines for humans are alum-precipitated cell-free filtrates of *Bacillus anthracis*. In the case of the U.S. vaccine, this precipitate is adsorbed onto aluminum hydroxide. The aluminum hydroxide adjuvant is included in the vaccine preparation to boost the immune response to the PA. Aluminum hydroxide is used in many vaccines and is thought to stimulate humoral rather than cell-mediated immunity (Ivins et al., 1998). The culture filtrates are processed to maximize the content of PA. The cell-free filtrate is primarily PA but also contains EF, LF, and other contaminants from culture (Ivins et al., 1998; Miller et al., 1998). PA vaccine for humans also elicited antibody production to EF and LF in rats and guinea pigs (Ivins et al., 1986; Turnbull et al., 1986; Ivins, 1988), suggesting that the contamination is sufficient to elicit a biological response.

The primary goal of anthrax vaccination is to produce neutralizing antibodies to PA. Subsequent exposure to anthrax infection would then eliminate the pathogenic potential of *B. anthracis* by eliciting the production of antibodies that neutralize PA. Without PA, EF and LF are incapable of acting as virulence factors. Barnard and Friedlander (1999) vaccinated guinea pigs with several different live recombinant *Bacillus anthracis* strains (pX01–, pX02–) that each produced a different amount of PA without producing the capsule, EF, or LF. The protective effect of these strains correlated with the production of PA and with the anti-PA antibody titer elicited in vivo. Studies by Turnbull and colleagues (1988) and Ivins (1988) in guinea pigs have provided evidence that PA is an essential component of the vaccine and that protection against anthrax in guinea pigs is possible in the absence of any detectable antibody to LF or EF. However, some studies have suggested that the protective effect of anthrax vaccine does not necessarily correlate with the antibody titer to PA in vivo. Studies by Little and Knudson (1986) indicated that a high titer to PA did not necessarily reflect the level of expected protection from infection. Studies by Turnbull and colleagues (1988) suggested that it is also important for the PA antigen to be presented to the immune system in such a way as to stimulate more than just a humoral immune response. Challenge tests with aerosol anthrax spores have shown that the Sterne live spore vaccine was more efficacious than PA-based

vaccines (Ivins, 1988), suggesting that cellular immunity as well as humoral immunity is important for protection against anthrax infection.

Animal Studies

As noted earlier in this chapter, adverse health outcomes in animals after injection may result from the toxic effects of the injected substances or from stimulation of the immune system. Injection of the live spore vaccine can cause infection. Injection of protective antigen vaccines can result in adverse effects associated with administration of the exotoxins.

Live attenuated spore vaccines. *Studies in veterinary use.* Many of the studies using the live spore vaccine have involved large-scale vaccination of animals of economic importance. Avirulent, nonencapsulated strains of *B. anthracis,* including the Sterne live spore vaccine, have been used for vaccination. In general, these studies have relied on anecdotal reports from farmers and veterinarians to measure the incidence of reactions of livestock (primarily horses, cows, calves, sheep, lambs, and goats) to the vaccination. In some cases, data on the number of animals vaccinated and the number of deaths from anthrax have been collected by survey or from veterinarian reports.

In most cases, the primary focus has been on evaluating the efficacy of the vaccine, rather than on monitoring adverse effects; therefore, many studies of veterinary use of the live spore vaccine have not commented on adverse effects associated with vaccination (Sterne et al., 1942; Kaufmann et al., 1973; Salmon and Ferrier, 1992).

The primary health outcomes in animal studies are edema at the site of injection, a febrile response, or death. The extent of the edema ranged from no reaction, to mild irritation, to lameness in some animals. Edema is due to elaboration of the edema toxin or to an allergic response to a previously administered vaccine. An early study by Sterne (1939) used a retrospective questionnaire to solicit complaints regarding the effectiveness and adverse effects of vaccinating sheep and cattle with live spore vaccines. With a limited response to the questionnaire, the majority of reports for cattle indicated that no reactions occurred. However, some cattle experienced lameness and transient decreases in milk yield. Some animals showed severe swelling at the site of injection and one death occurred. Kolksov and Mikhailov (1959) described either insignificant or mild reactions in the majority of 650,000 animals that were vaccinated. Some horses and cattle had swelling at the injection site measuring 12–40 cm^2 and lasting 3 to 4 days, along with a slight temperature rise. Of the 650,000 animals (including cattle, horses, oxen, sheep, and goats) 20 animals were reported as dying from unspecified causes. In another study of the Sterne live spore vaccine, it was noted that three of the 34,000 cattle, horses, mules, hogs, and sheep receiving the vaccine experienced instances of excessive swelling (Lindley, 1963). Increased temperature of the animals, lasting for days, has been observed in other studies (Kolksov and Mikhailov, 1959; Kolosov and Borisovich, 1968;

Kolesov et al., 1968). Tanner and colleagues (1978) reported a febrile period lasting less than 24 hours in 12 of 49 vaccinated cows but no change in daily food consumption. In addition, decreased activity or decreased milk production has been noted and is presumed to be due to the presence of inflammation. Studies of live spore vaccine in veterinary use are primarily descriptive, so the actual incidence of adverse reactions to the vaccine is not known. The committee did not find any long-term studies (greater than a year) that monitored adverse effects from vaccination with the live spore vaccine.

Studies in laboratory animals. Studies with the live spore vaccine in laboratory animals have been conducted under better controlled conditions than those in veterinary practice. Many of these studies make no mention of adverse effects in guinea pigs, hamsters, rabbits, or mice (Klein et al., 1962; Jaiswal and Mittal, 1979; Ezzell and Abshire, 1988; Turnbull et al., 1988; Stepanov et al., 1996). Small laboratory animals such as rabbits, guinea pigs, and mice are more susceptible than larger animals to dying from administration of the live spore vaccine (Welkos, 1987; Welkos and Friedlander, 1988; Ivins et al., 1990). High doses of the live spore vaccine killed one-third of the guinea pigs studied by Turnbull and colleagues (1986, 1988) and up to 60 percent of the guinea pigs in a study by Klein and colleagues (1962).

A thorough study by Gusman and Migulina (1967) histologically examined rabbits and guinea pigs immunized with live anthrax spore vaccines by subcutaneous injection. They monitored tissue from internal organs as well as from the site of administration of the vaccine for up to 210 days after vaccination. Edema occurred at the site of the injection, sometimes with hemorrhage and abscess formation. Dilation of the blood vessels and infiltration of the site with segmented white blood cells also occurred. Edema and inflammation lasted for 14 days, followed by the formation of granulation tissue. Over a 2-month period, researchers noted changes in the lymphoid organs, consistent with a response to an antigen. In addition, histological changes were evident in the liver and heart muscle but resolved within 2 months.

Vaccination with live spore vaccine may also lead to complications of otherwise symptomless infections or to death from anthrax when the animal is subject to trauma. Kolesov and Gutiman (1968) and Stefanova (1968) noted that rabbits injected with live spore vaccine would sometimes die. They confirmed by autopsy and bacteriological examination that the rabbits died from pasteurellosis infection, not from anthrax. Thus, vaccination with the live spore anthrax vaccine may activate an underlying infection in a rabbit that was in satisfactory health prior to vaccination. In a similar manner, death from anthrax may occur more readily in an animal whose health is compromised. Stefanova (1968) found that rabbits subjected to the trauma of an ear biopsy after vaccination with the live spore vaccine were more likely to die than animals not subject to the trauma.

Guinea pigs vaccinated with the live spore vaccine have delayed-type hypersensitivity reactions 1 year after vaccination (Shlyakhov, 1970; Shlyakhov and Rubinstein, 1994b), indicating stimulation of the cellular immune response.

These studies have employed anthraxin, an incompletely defined antigen used for skin testing. A positive delayed-type hypersensitivity reaction to anthraxin was associated with hyperemia of 64 mm^2 and a twofold thickening of the skin.

Protective antigen-based vaccines. Protective antigen vaccines have had little use in veterinary practice but have been tested in laboratory animals either with or without adjuvant. The primary goal of these studies has been to determine the efficacy of injections of PA, with or without adjuvant, in protecting against infection with *Bacillus anthracis.* In addition, the studies have attempted to correlate the presence of antibodies with the degree of protection afforded by the vaccination.

Most studies in laboratory animals with the protective antigen vaccine have not mentioned adverse effects associated with vaccination. Many studies conducted in guinea pigs employed different vaccination regimens using culture filtrates of PA with and without alum adjuvant. Reports of these studies did not mention adverse effects (DeArmon et al., 1961; Klein et al., 1961; Puziss and Wright, 1963; Gulrajani et al., 1968; Little and Knudson, 1986; Ezzell and Abshire, 1988; Ivins, 1988; Ivins et al., 1986, 1989, 1990, 1993, 1994, 1995; McBride et al., 1998). In addition, reports of studies with similar vaccinations in mice and rabbits did not mention adverse events after vaccination (Wright et al., 1954; DeArmon et al., 1961; Puziss and Wright, 1963; Gulrajani et al., 1968; Ivins et al., 1990). The lack of data in these instances can indicate that no adverse effects occurred, that adverse events were not monitored, or that adverse events were not sufficiently severe to warrant termination of the experiment. The primary purpose of most of the studies was to evaluate the effectiveness of the vaccine against *Bacillus anthracis* infection. In general, most of the studies monitored animals for 1 to 2 months. A few studies extended to 1 or 2 years.

In a study by Wright and coworkers (1954), 25 rabbits received five 0.5-ml intracutaneous injections of anthrax vaccine on alternate days. The rabbits were sacrificed 23 days later. Complete autopsies, including gross and microscopic examination of all organs, revealed no adverse effects. Limited studies have also been conducted in nonhuman primates. A study in rhesus monkeys using the licensed anthrax vaccine revealed no remarkable local or systemic reactions (Ivins et al., 1998). Darlow and colleagues (1956) vaccinated 30 rhesus monkeys with the alum precipitate of the PA antigen and found no evidence of toxicity in the 10 animals that were monitored for 2 years. In this study, one control and one vaccinated animal developed a transitory illness and recovered within 3 days. The authors attributed the illness to a transitory infection, unrelated to the anthrax study. Three of the immunized animals monitored for the 2 years were reported to have died from other causes during the experimental period. Darlow also found that 50 ml of the vaccine preparation injected intravenously into rabbits resulted in no deaths and no apparent adverse effects. Wright and coworkers (1954) injected five monkeys with the PA antigen and did not mention any adverse effects. A booster injection given 3 months later did not cause significant local reactions or lesions observable on autopsy 3 weeks later.

Recombinant protective antigen-based vaccines. No adverse effects were reported after vaccination of mice or guinea pigs with either *Bacillus subtilis* expressing recombinant PA (Welkos, 1987; Welkos and Friedlander, 1988; Miller et al., 1998) or DNA encoding for PA (Gu et al., 1999). Using recombinant techniques, Singh and colleagues (1998) generated a noncleavable PA mutant that bound to the receptor with an affinity equal to that of native PA but failed to bind LF or EF. The authors did not comment on adverse effects when they used the mutant PA to vaccinate guinea pigs (Singh et al., 1998).

Protective antigen-based vaccines with miscellaneous adjuvants. In an attempt to increase the effectiveness of PA vaccines, investigators have employed different adjuvant preparations in combination with PA. Ivins and coworkers (1992) immunized mice with PA and a bacterial cell wall adjuvant preparation. This combination occasionally resulted in a small, nonnecrotic granuloma. A later study by Ivins and colleagues (1995) used many combinations of adjuvants with PA. The authors reported adverse effects only when they used purified PA combined with all of the following: squalene, Tween, Pluronic block copolymer L121, and threonyl muramyl dipeptide. Five of the twenty animals immunized with this combination died within 1 to 5 days. The cause of death was unknown, but significant vascular congestion in multiple organs suggested that the animals suffered from shock and cardiovascular collapse. Other adjuvants (including other combinations that included squalene) provided adequate protection and did not elicit adverse reactions.

Conclusions on Animal Studies

Few meaningful conclusions for humans can be drawn from animal studies of the anthrax vaccine. Many of the animal studies have used the anthrax live spore vaccine and therefore, have limited applicability for evaluating the toxicity of protective antigen vaccines. Additionally, animal studies using the PA vaccine typically have reported only on short-term local reactions to injection of the vaccine. Further, most of the studies do not indicate whether the authors monitored for adverse consequences of vaccination.

Human Studies

The committee used only the peer-reviewed literature to form its conclusions on the weight of the evidence for associations of the anthrax vaccine with adverse health effects. Only a few published peer-reviewed studies have examined potential adverse effects of the anthrax vaccine when administered to humans. In considering the need for future research, the committee evaluated other studies in addition to peer-reviewed publications.

Live Attenuated Spore Vaccine Studies

The committee examined information on the human studies and extensive field trials conducted in the Soviet republics from the 1940s to the 1970s (described in Shlyakhov and Rubinstein, 1994a). These Soviet studies used the live spore anthrax vaccine, which differs substantially from the protective antigen anthrax vaccines used in the United States and the United Kingdom, in terms of composition, reactogenicity, and potential residual virulence. Moreover, the Soviet studies performed neither passive nor active long-term monitoring. For these reasons, the committee did not include the live spore vaccine studies in its analysis.

The committee notes a recent literature review on anthrax vaccine studies (Demicheli et al., 1998) conducted according to Cochrane Collaboration guidelines for systematic reviews of health care interventions. Only the Brachman study (described below) met the Cochrane criteria for prospective randomized or quasi-randomized studies of a protective antigen vaccine.[5]

Healthy-Volunteer Studies

During the development of the anthrax vaccine, several early studies examined adverse reactions in humans but did not provide detailed information on the nature of the monitoring for adverse effects. These studies used early versions of the culture filtrate (protective antigen) vaccine. Wright and colleagues (1954) described the reactions of 660 persons at Camp Detrick who received a total of 1,936 injections. They found that 0.7 percent of vaccinated subjects reported systemic reactions—typically consisting of mild muscle aches, headaches, and mild-to-moderate malaise lasting 1 to 2 days. Significant local reactions—typically swelling (5–10 cm in diameter) and local pruritus (itching)—occurred in 2.4 percent of the subjects. The incidence of local reactions increased with the number of injections.

In another study at Fort Detrick (Puziss and Wright, 1963), 0.5-ml injections of protective antigen led to similar results. The study reported low rates of erythema, edema, or pruritus at the site of injection (no details were provided) and no systemic reactions.

Darlow and colleagues in Great Britain (1956) reported on the administration of 1,057 injections of the anthrax vaccine to 373 individuals (369 persons received two or more injections) over a period of 4 years. Most of the reactions were mild and brief (local tenderness and swelling). There was an increase in the number of persons experiencing pain after the second dose, and local reactions

[5]The one live spore vaccine study that met the Cochrane criteria was a field trial conducted by Burgasov and colleagues. In this study, 107,285 individuals received the live spore anthrax vaccination and 49,974 individuals served as controls. The review by Demicheli and colleagues (1998) does not report any information from the Burgasov study regarding adverse effects of the vaccine.

increased with successive booster injections. The study reported that three people had brief and mild fever.

Brachman Study

Brachman and colleagues (1962) conducted the only randomized clinical trial of vaccination with a protective antigen anthrax vaccine. Although the vaccine used in this study was similar to the current vaccine (used to immunize Gulf War troops and currently available in the United States) in that it was a PA vaccine, the manufacturing process has since changed and a different strain of anthrax bacillus is now used (GAO, 1999c).

The clinical trial was conducted among 1,249 eligible workers[6] at four goat hair processing mills in which some raw materials were contaminated by anthrax bacilli. Both cutaneous and airborne anthrax were endemic; approximately one case of anthrax occurred per 100 employees per year in these mills. After the initial series of three injections, the study had to be terminated at the largest mill, which employed nearly half of the subjects, because of an outbreak of inhalation anthrax that required the vaccination of all employees. At the remaining mills, 480 participants completed the series of injections (230 of whom were randomized to receive active vaccinations and 250 to receive placebo injections) and 81 participants did not complete the series.[7] The study subjects did not know whether they had received the active vaccine or placebo; the article does not state whether the investigators were also blinded to the allocation.

The report of the study does not always clearly state whether the results in the three mills apply only to the 480 subjects who completed the vaccination series or also included results from the 81 subjects who did not complete the series. Neither does it state whether the results apply only to the 480 subjects in the three mills who completed the series or whether the results include the subjects from the largest mill who had been randomized, received the initial injections, and were partially evaluated prior to the decision to withdraw the mill's employees from the study.

Participants were examined 24 and 48 hours following each vaccination to assess both local and systemic reactions to the vaccine. There were no reports of subsequent active or passive surveillance for possible adverse effects beyond 48 hours after each vaccination (there was further monitoring for the vaccine's efficacy).

Of the 230 actively vaccinated subjects who completed the inoculations, one individual (0.4 percent) developed anthrax. Of the 250 individuals receiving placebo, 12 (4.8 percent) developed anthrax. The great majority of cases of an-

[6]Employees who had a previous case of anthrax were not eligible for the study. Of the 1,249 employees eligible for participation, 340 refused to participate in the study.

[7]The authors state that there was a gradual decline in participation in the study, partly because of changes in the nature of the textile business and partly because some of the employees withdrew from the program. Reasons for withdrawal were not stated.

thrax were of the cutaneous type; there were not enough cases of inhalation anthrax to determine if vaccination was effective against this, the most lethal form of anthrax.

The typical reaction was described as a ring of erythema (1–2 cm in diameter) at the injection site, with local tenderness that lasted 24–48 hours. Some individuals (the authors did not report the number) reported more extensive edema, erythema (>5 cm in diameter), pruritus, induration, and/or small painless nodules at the injection site (lasting up to several weeks). Twenty-one persons had moderate local edema that lasted up to 48 hours. Three individuals had edema extending from the deltoid to the mid-forearm (in one case, to the wrist) that dissipated within 5 days. Systemic reactions occurred in two individuals (0.9 percent of the actively immunized subjects) who experienced "malaise" lasting 24 hours following vaccination. The study notes that three individuals who received the placebo (0.1 percent alum) had mild reactions.

Other Studies and Information

Several other studies, discussed below, had information on the safety of the anthrax vaccine, but these studies have not been published in the peer-reviewed literature and were not considered in the committee's conclusions regarding the strength of the evidence for associations with adverse health outcomes. Publication of these studies would substantially increase the body of information needed to form conclusions regarding health effects of the anthrax vaccine. Most of the studies are currently described only in secondary sources (e.g., reviews, congressional testimony, General Accounting Office [GAO] reports). Additionally, a recently published article in the CDC *Morbidity and Mortality Weekly Report* (CDC, 2000) provided summary results of several of the studies. A few of the studies have only recently been completed or are ongoing.

Investigational new drug data. In the early 1970s, CDC submitted data on the safety of the anthrax vaccine in support of an application to license the vaccine. The committee did not have access to primary data but examined the information provided in secondary sources (Friedlander et al., 1999; GAO, 1999b,c,d). At the end of its study, after the committee had completed its work, the committee received the IND information that had been requested earlier through a FOIA (Freedom of Information Act) request. Another Institute of Medicine committee that is currently studying the safety and efficacy of the anthrax vaccine will be able to examine this information.

The IND data included information on the reactions of approximately 7,000 individuals who had received approximately 16,000 doses of the vaccine (four lots manufactured by MDPH). With active monitoring (there was no description of the monitoring methods), mild local reactions (≤3 cm) occurred in 3–20 percent of all doses, moderate local reactions (>3 to <12 cm) in 1–3 percent of all doses, and severe reactions (≥12 cm) in less than 1 percent of doses. Four indi-

viduals reported transient systemic reactions consisting of fever, chills, nausea, and body aches (Friedlander et al., 1999).

VAERS reports. As described earlier in this chapter, the Vaccine Adverse Event Reporting System is a passive surveillance system consisting of reports filed by health care providers, individuals receiving vaccinations, family members, or others. As noted earlier, VAERS data are useful as a sentinel for adverse events but are limited in their usefulness for assessing the rate of adverse events since underreporting is likely and the information may be incomplete or duplicative, or may not always have been confirmed by medical personnel (IOM, 1994).

The committee reviewed summaries of VAERS data but did not review the individual VAERS forms. From its inception in 1990 through July 1, 1999, there have been 215 VAERS reports regarding anthrax vaccination (Ellenberg, 1999). The majority of the reports describe local or systemic symptoms including injection site edema, injection site hypersensitivity, rash, headache, and fever. Twenty-two of the VAERS reports are considered serious events[8] and were described as occurring (or being diagnosed) from 45 minutes to 4½ months after receiving the vaccination. Reports of serious events include five patients hospitalized with severe injection site reactions, one individual with a widespread allergic reaction, one individual with a case of aseptic meningitis 9 days after vaccination, two individuals who experienced Guillain-Barré syndrome, one individual diagnosed with bipolar disorder 3 weeks after receiving the vaccine, one individual with an onset of multi-focal inflammatory demyelinating disease, and one individual who experienced the onset of lupus (Ellenberg, 1999). In recent congressional testimony FDA stated, "None of these events, except for the injection site reactions, can be attributed to the vaccine with a high level of confidence, nor can contribution of the vaccine to the event reported be entirely ruled out ... . [T]he reports on anthrax vaccine received thus far do not raise any specific concerns about the safety of the vaccine" (Ellenberg, 1999). An external review panel, the Anthrax Vaccine Expert Committee, has recently been established by the Department of Health and Human Services at the request of the Department of Defense (DoD) to review each VAERS report received regarding anthrax vaccination.

Special Immunization Program Safety Study. Since 1973, 1,590 workers at the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) have received 10,451 doses of the anthrax vaccine (Claypool, 1999; Friedlander et al., 1999). Visits to the occupational health clinic were used as a method of collecting and passively monitoring information on adverse reactions. Four percent of doses resulted in a local reaction (redness, induration, itching, or edema) at the site of injection. Systemic reactions (headache, fever, chills, malaise, muscle and joint aches) occurred with 0.5 percent of doses. Individuals received annual physical

[8]Adverse events resulting in life-threatening illness, hospitalization, permanent disability, extended hospital stay, or death (Ellenberg, 1999).

exams. Diseases and unexplained symptoms occurred at a rate that would be expected in a comparable group (Claypool, 1999).

Fort Bragg Booster Study. Pittman and colleagues (1997) studied the frequency of possible vaccine-related adverse outcomes during an open-label study of DoD personnel who received anthrax and/or botulinum toxoid vaccines during Operation Desert Shield/Desert Storm in 1990–1991. The objectives of the study were to assess the persistence of antibodies to the vaccines, determine the serological response 30 days after receiving a booster dose, and evaluate the reactogenicity of the vaccines. All of the 486 subjects were male volunteers who had documented records of receiving one or more doses of the anthrax or botulinum vaccine during the Gulf War. Subjects received booster shots (in different arms) of either the anthrax or the botulinum toxoid vaccine, or both, depending on what they had received in 1990–1991. The report states that there was daily monitoring for systemic and local reactions but does not state the total duration of follow-up.

Approximately 20–25 percent of subjects complained of erythema, induration, or swelling at the site of the anthrax vaccine injection; about 3 percent described the reaction as severe. Fever (defined as an oral temperature of 100.5°F or greater) occurred in 2.8 percent of subjects. Systemic symptoms within the first 7 days after receiving the vaccine(s) occurred in 44 percent of study subjects. Symptoms included muscle aches (30 percent), headache (16.5 percent), feeling ill (16 percent), and rash (16 percent). Other symptoms included loss of appetite, difficulty breathing, joint aches, and nausea. The study reports that most of the symptoms were mild; however, 20 volunteers had severe symptoms (the authors did not define "severity").

Canadian Armed Forces Study. A study monitored 547 individuals in the Canadian Armed Forces who received the anthrax vaccine in 1998 (Claypool, 1999; Friedlander et al., 1999). Mild local reactions occurred with 10.1 percent of doses, moderate local reactions occurred with 0.5 percent of doses, and there were no reports of severe local reactions. Systemic reactions occurred with 1.5 percent of doses (five individuals had fever, two had heartburn, one experienced a "transient nerve disorder") (Claypool, 1999). Reactions were transient except for one individual who reported persistent nodules. The type of monitoring for adverse health effects was not described.

USAMRIID Reduced Dose and Route Change Study. A pilot study at USAMRIID compared the safety of three doses of anthrax vaccine delivered by subcutaneous injection at 0, 2, and 4 weeks (the current primary schedule and route) with two doses given subcutaneously, and with two intramuscular deltoid injections (Claypool, 1999); 173 people were studied. The incidence of systemic effects did not differ between the three groups: headache (14 percent), malaise (9 percent), loss of appetite (3 percent), nausea or vomiting (3 percent), muscle ache (3 percent), itchiness (3 percent), and low-grade fever (3 percent). Local reactions

(e.g., redness and swelling at the injection site, subcutaneous nodules) occurred more frequently with subcutaneous than with intramuscular injections (5–7 percent). Male vaccine recipients reported local reactions less frequently after subcutaneous injections (5–32 percent) than female vaccine recipients (39–66 percent). The report did not describe the type of monitoring for adverse health effects.

Tripler Army Medical Center Survey. A self-administered questionnaire was used to collect data on 603 health care personnel who received the anthrax vaccine at Tripler Army Medical Center beginning in September 1998 (CDC, 2000). As reported in congressional testimony, the survey found a high incidence of local transient reactions (70 percent with subcutaneous nodules and 65 percent with muscle soreness) (Claypool, 1999). Muscle aches, the most frequently reported systemic complaint, were reported in 15 percent of vaccine recipients. Three VAERS reports were submitted on the individuals in this study, and one individual lost more than a day of work; there were no hospitalizations. Gender differences in the number of reactions have been noted in this study. A higher proportion of women reported outpatient visits (e.g., after the second dose, 2.0 percent of males and 13.8 percent of females reported making outpatient visits) and local reactions (e.g., after the second dose, 20.4 percent of males and 46.9 percent of females reported moderate to severe redness) (GAO, 1999d).

Additional studies. The U.S. Air Force is completing a study comparing visual acuity in 354 vaccinated aircrew members with 363 aircrew personnel who were not vaccinated against anthrax. Preliminary analysis reported in congressional testimony indicates that changes in visual acuity occurred in 12 percent of vaccinated and 16 percent of unvaccinated crew members during the course of a year (Claypool, 1999).

Service members stationed in Korea completed a mandatory questionnaire when they reported for anthrax vaccination. Questions included the service member's reaction to the previous dose of the anthrax vaccine. Data from 6,879 questionnaires noted gender differences in the reported rate of transient adverse reactions, with higher rates in women. After the first or second dose of the vaccine, 82 (1.9 percent) of 4,348 men and women reported limited effects on their work performance, 21 (0.5 percent) went to the clinic for evaluation, and 1 required hospitalization for an injection site reaction (CDC, 2000).

Conclusions on Human Studies

There is a paucity of published peer-reviewed literature on the safety of the anthrax vaccine. The committee located only one randomized peer-reviewed study of the type of anthrax vaccine used in the United States (Brachman et al., 1962). However, the formulation of the vaccine used in that study differs somewhat from the vaccine given to Gulf War veterans (and currently in use). The Brachman study (and other early experimental studies) found transient local and systemic effects (primarily erythema, edema, induration) of the anthrax vaccine.

There was no long-term monitoring for adverse outcomes. The committee did not compare the incidence of transient effects with other vaccines.

Studies of the anthrax vaccine have not used active surveillance to systematically evaluate long-term health outcomes. This situation is unfortunately typical for all but a few vaccines. The committee strongly encourages active monitoring to evaluate the long-term safety of the anthrax vaccine.

To date, published studies have reported no significant adverse effects of the vaccine, but the literature is limited to a few short-term studies. Reviewing the large body of results that have not yet been published would enable more definitive conclusions about the vaccine's safety. The committee strongly urges investigators conducting studies on the safety of the anthrax vaccine to submit their results to peer-reviewed scientific journals for publication.

The committee's findings are best regarded as an early step in the complex process of understanding the safety of the anthrax vaccine, which began with the vaccine's licensure in 1970 and the 1985 FDA advisory panel finding that categorized the vaccine as safe and effective. Active long-term monitoring of large populations will provide further information for documenting the relative safety of the anthrax vaccine.

The committee concludes that there is sufficient evidence of an association between anthrax vaccination and transient acute local and systemic effects (e.g., redness, swelling, fever) typically associated with vaccination.

The committee concludes that there is inadequate/insufficient evidence to determine whether an association does or does not exist between anthrax vaccination and long-term adverse health effects.

The latter finding means that the evidence reviewed by the committee is of insufficient quality, consistency, or statistical power to permit a conclusion regarding the presence or absence of an association between the vaccine and a health outcome in humans.

BOTULINUM TOXOID

Botulinum toxins, known primarily for causing cases of foodborne botulism,[9] are produced by the anaerobic bacterium *Clostridium botulinum*. The organism itself is not thought to play a role in the poisoning syndrome (Middle-

[9]Botulism is a paralytic disease with three primary clinical manifestations: foodborne, wound, and infant (Sellin, 1984; Fauci et al., 1998). Incidence of the disease is low, fewer than 100 cases of botulism are reported in the United States each year (Lopez, 1998). Ingestion of botulinum spores is the primary exposure pathway. A trivalent equine antitoxin is available from CDC for the treatment of foodborne botulism. A heptavalent antitoxin is currently in IND status (Franz et al., 1997).

brook et al., 1994). However, different strains of the bacillus produce seven distinct botulinum toxins (A–G). These toxins are among the most toxic compounds per body weight of agent, with an LD_{50} of 0.001 μg/kg in mice (for comparison, the LD_{50} of sarin in laboratory mice is 100 μg/kg) (USAMRIID, 1996). It is interesting to note that botulinum A toxin is successfully used to relax muscular spasms for a number of therapeutic purposes.[10] The doses used are so minute that they do not produce toxic reactions, nor are they immunogenic.

Work on modifying the botulinum toxin to the nontoxic form of a toxoid began in 1924.[11] Experimental work on a toxoid for use in humans was first reported in the 1930s by the Russian scientist Velikanov (Anderson and Lewis, 1981; Middlebrook and Brown, 1995). A bivalent toxoid (for serotypes A and B) was developed in the United States in the 1940s. Further research led to a pentavalent toxoid (serotypes A–E) first produced in large lots by Parke, Davis, and Company in 1958 under contract to the U.S. Army (Anderson and Lewis, 1981). CDC submitted an Investigational New Drug application for the pentavalent toxoid in 1965 (IND 161; Rettig, 1999). In the 1970s, the Michigan Department of Public Health (now BioPort Corporation) produced the pentavalent toxoid.

Currently, the toxoid is in IND status. As described below, the toxoid has been administered to volunteers for testing purposes and to occupationally at-risk workers. Additionally, it is estimated that 8,000 U.S. troops received the toxoid during the Gulf War. Under an FDA Interim Rule (U.S. DHHS, 1990), the FDA commissioner was given the authority to waive IND requirements (e.g., informed consent) in specific military exigencies (Rettig, 1999).

The schedule for the pentavalent toxoid calls for subcutaneous injections at 0, 2, and 12 weeks, followed by annual boosters. Contraindications for administering the vaccine include alum, formaldehyde, or thimerosal sensitivities or hypersensitivity after receiving a previous dose (USAMRIID, 1996). Recent advances in molecular cloning techniques and new knowledge about the molecular mechanisms of action of the toxins have opened up avenues for new botulinum vaccine development (Middlebrook, 1995).

[10]The primary use has been to reduce muscle spasm by blocking the release of acetylcholine at the neuromuscular junction. This is used in the treatment of strabismus, hemifacial spasm, cervical dystonia, and other spastic disorders (Cardoso and Jankovic, 1995; Tsui, 1996). Transient local muscle weakness can occur; however, no hypersensitivity reactions have been reported (Tsui, 1996). Systemic effects such as fever, malaise, fatigue, and flulike symptoms have been observed but in one double-blind study occurred less frequently than when placebo was given (Tsui, 1996).

[11]A toxoid is a modified bacterial toxin that is made nontoxic but has the capacity to stimulate the formation of antitoxin antibodies (Fauci et al., 1998).

Toxicology

Mechanism of Action

Knowledge of the pathogenic mechanisms of *Clostridium botulinum* can provide insight into the potential adverse effects associated with administration of the botulinum toxoid vaccine. The signs and symptoms of botulism are due to the action of neurotoxins that are synthesized during cell growth of *C. botulinum* and released prior to or after lysis of the bacteria.

There are seven immunologically distinct types of botulinum neurotoxins: types A through G. Human botulism is caused principally by types A, B, E, and F toxins, and animal botulism is principally caused by types C and D (Sellin, 1984). In horses, the *Clostridium botulinum* that colonizes the intestinal tract produces type B toxin, causing the symptoms of shaker foal syndrome.

The mechanism of action of botulinum neurotoxins has recently been reviewed (Simpson, 1989; Brin, 1997). The seven neurotoxins are all metalloenzymes that cleave various components of the proteins involved in the release of the neurotransmitter, acetylcholine. Botulinum toxins share a number of structural features with tetanus toxins, even though the clinical symptoms of poisoning are quite different. The toxin molecule has three functional domains. The carboxy terminal portion of the molecule mediates binding of the toxin to the presynaptic nerve terminal at the neuromuscular junction. The central third of the molecule acts to internalize the neurotoxin inside the nerve ending. After internalization and disulfide cleavage, the light chain or amino terminal section then translocates the toxin to the cytosol where it inhibits the binding of synaptosomal vesicles to the axon terminal membrane, thus inhibiting the release of acetylcholine. Inhibiting the release of acetylcholine significantly affects nerve transmission between motor nerves and the voluntary muscles, causing paralysis and loss of respiration, a process that occurs at doses lower than required to affect the autonomic nervous system. All ganglionic synapses require acetylcholine and thus are disrupted. In addition, the postganglionic parasympathetic nervous system requires acetylcholine. The clinical effect of the toxin is thought to be due primarily to its effects on the peripheral nervous system because the botulinum neurotoxin does not cross the blood–brain barrier (Simpson, 1993). Extreme cases of poisoning with botulinum toxin result in total paralysis, with the patient incapable of moving or breathing.

To produce the toxoid, toxins are partially purified from culture supernatants and exposed for prolonged periods to formaldehyde. After exposure to formaldehyde they are tested for toxicity in mice and guinea pigs to ensure that the neurotoxin was inactivated.

Animal Studies

As noted earlier in this chapter, adverse health outcomes can result either from toxic effects of the injected toxoid preparation or from stimulation of the immune system. Toxic effects of the vaccination itself could be due to traces of

formaldehyde or preservative in the toxoid preparation, contaminant proteins in the toxoid preparation, toxin that has not been inactivated by formaldehyde, and/or the adjuvant.

Studies in veterinary use. *Botulinum toxoids type C and D.* Animal botulism is caused principally by the type C and D neurotoxins. In veterinary use, vaccination of mink and ferrets (Pranter, 1976; Shen et al., 1981), chickens (Dohms et al., 1982; Kurazono et al., 1985), pheasants (Kurazono et al., 1985), and cattle (Tammemagi and Grant, 1967) has been reported using either monovalent type C toxoid or bivalent types C and D. These studies did not mention adverse effects from vaccination. In the study using type C toxoid in chicks (Dohms et al., 1982), survivors of the toxin challenge showed no lesions at the site of vaccination. In addition, Davidson (1976) reviewed the use of types C and D toxoids for veterinary purposes and did not mention adverse effects due to vaccination. The primary purpose of most of the studies was to evaluate the effectiveness of the vaccine against challenge with the botulinum neurotoxin, not to evaluate adverse effects. Most of the studies monitored animals for 1–2 months.

Botulinum toxoid type B in veterinary practice and laboratory animals. Shaker foal syndrome is a neuromuscular condition affecting 2- to 8-week-old foals. Administering type B botulinum toxoid can mimic the symptoms of shaker foal syndrome. Swelling at the injection site has been reported in four horses administered two doses of type B botulinum toxoid (Thomas et al., 1988). These swellings were hard and approximately 75 cm^2 in area. The report did not state if the swelling occurred with the first, second, or both doses of the toxoid.

Studies in laboratory animals. Studies with botulinum toxoids have been done in guinea pigs, rabbits, and mice with type E (Kondo et al., 1969), type A (Gendon, 1958), and pentavalent types A–E toxoid (Cardella, 1964); in mice with type F toxoid (Mikhailova, 1966); and in guinea pigs with types C and D toxoid (Mathews, 1976). In none of these studies did the authors mention adverse effects, which may indicate that no adverse effects occurred, that adverse events were not monitored, or that the adverse events were not sufficiently severe to warrant termination of the experiment.

Studies in guinea pigs suggest that skin-sensitizing anaphylactic antibodies may be produced in response to the administration of a combination of type B botulinum toxoid with the complex typhoid antigen (Yefremova, 1980). Such skin-sensitizing antibodies in the guinea pig are associated with immediate hypersensitivity reactions and respiratory allergy. Effects of administration of type B toxoid alone were not investigated in this study.

Recombinant DNA methods have been used to generate fragments of the botulinum neurotoxins, in hopes of developing a molecule without neurotoxicity but able to provoke an immune response and protect against botulinum neurotoxin activity. Such fragments of types A, B, and C botulinum neurotoxin have been generated and tested for toxicity and immunogenicity in mice (Middle-

brook et al., 1994; Whalen et al., 1996; Kiyatkin et al., 1997; Bavari et al., 1998; Byrne et al., 1998; Smith, 1998). The monitoring period for adverse effects in these animal studies was no more than months. The authors of these studies did not mention adverse effects from vaccination with toxin fragments. Clayton and colleagues (1995) expressed a fragment of botulinum neurotoxin type A in *Escherichia coli*. Initial experiments immunized mice with a crude extract of *E. coli* expressing the gene of interest; several mice died following the second or third vaccination. The authors suspected endotoxin contamination. Subsequent experiments used purified neurotoxin fragments, and no toxicity occurred after repeated vaccination of these recombinant preparations.

Conclusions on Animal Studies

Animal studies using botulinum toxoid vaccines have reported minimal transient local reactions and swelling at the injection site. Only short-term outcomes were reported, and in most studies, the reports did not mention adverse effects.

Human Studies

Only a few published peer-reviewed studies have examined the potential adverse health effects of the botulinum toxoid vaccine when administered to humans. The committee based its conclusions about possible associations between the toxoid and health effects solely on the peer-reviewed literature and included other studies when assessing research needs.

Published Studies of Laboratory Workers

Early studies of the initial univalent botulinum toxoids in the 1940s reported a significant number of local and systemic reactions (Middlebrook and Brown, 1995). Toxoids were further purified in the 1950s, and a study published in 1962 (Fiock et al., 1962) reported on tests of bivalent toxoid preparations by Parke, Davis, and Company. This study of laboratory personnel at Fort Detrick focused on the efficacy of four different schedules of bivalent botulinum toxoid vaccination. Personnel, not previously immunized with botulinum toxoid, were assigned to four different vaccination schedules as they reported for initial vaccination (50 individuals followed a 0-, 2-, 4-, and 6-week schedule; 25 individuals followed a 0- and 8-week schedule; 50 individuals followed a 0-, 2-, and 10-week schedule; and 25 individuals followed a 0- and 10-week schedule). The study reports that after 800 injections, no systemic or severe local reactions had occurred. Some individuals (the number is not reported) had a small subcutaneous nodule that lasted 2–3 weeks. The report does not discuss the surveillance methods for monitoring adverse effects.

A subsequent study by Fiock and colleagues (1963) examined four different pentavalent (A–E) toxoid lots prepared by Parke, Davis, and Company. This

study focused on the immunological response to pentavalent toxoids. In the first part of the experiment, 17 laboratory workers received 0.5-ml injections of a pentavalent toxoid on a 0-, 2-, and 10-week schedule. As a control group, additional personnel received a univalent toxoid on the same schedule (each univalent toxoid [A, B, C, D, or E] was given to five or six individuals). Four months later an additional 15 individuals received the same lot of the pentavalent toxoid on the same schedule. Satisfactory antitoxin titer levels were seen in these initial experiments. Groups of 30 individuals (the study does not indicate the number of groups or whether the individuals were new participants) were then immunized with one of the pentavalent toxoid lots on a 0-, 2-, and 12-week schedule, with a booster at 52 weeks. The only statement about adverse reactions made by the investigators in their report was that 400 individuals received the pentavalent toxoid with "no marked local or marked systemic reactions." Three persons had either a moderate local or moderate systemic reaction (the authors provided no details), and the authors stated that the incidence of mild local reaction was somewhat greater for the pentavalent toxoids than the control group toxoids (Fiock et al., 1962).

Other Studies and Reports

The committee received reports on several other studies, discussed below, with information on the botulinum toxoid vaccine. These studies have not been published in the peer-reviewed literature and were not considered in the committee's conclusions regarding the strength of the evidence for associations between botulinum toxoid and adverse health outcomes.

USAMRIID studies. *The Proceedings of the 1981 International Conference on the Biomedical Aspects of Botulism* (Lewis, 1981) details further studies on the pentavalent toxoids. Investigators at USAMRIID hypothesized that injections with formulations of the pentavalent botulinum toxoid containing less formaldehyde preservative would result in reduced pain at the site of injection. They conducted a full-series double-blind study to evaluate two formulations of the pentavalent toxoid with varying formaldehyde content; 36 previously nonimmunized laboratory workers received injections at 0, 14, and 84 days (13 individuals received the low-formaldehyde lot, 11 received the higher-formaldehyde lot, and 12 received the control toxoid [with an intermediate level of formaldehyde]). Reactions were recorded immediately and then at 24, 48, and 72 hours in all volunteers and after 72 hours as needed. The study reported no discernible differences in immediate pain. The group receiving the low-formaldehyde lot reported a slightly higher percentage of moderate local reactions (17.9 percent compared to 15 percent in those receiving the high-formaldehyde lot and 3 percent of controls). Moderate reactions were defined as edema or induration >30 mm and < 210 mm in any one diameter. No severe reactions occurred for any of the lots.

Fort Bragg Booster Study. Pittman and colleagues (1997) conducted an open-label study of DoD personnel 2 years after they had received anthrax and/or botulinum vaccines during Operation Desert Shield/Desert Storm in 1990–1991. As described above in the discussion of the anthrax vaccine, the objectives of the study were to assess the persistence of antibodies to the vaccines, to determine the serological response 30 days after receiving a booster dose (subjects received boosters for either the anthrax or the botulinum vaccine or both, depending on what they had received in 1990–1991), and to evaluate the reactogenicity of the vaccines. Of the 459 volunteers who received booster injections of the anthrax and botulinum vaccines, 13 percent reported erythema, and 15 percent noted induration at the site of the botulinum toxoid injection. The report states that the local reactions measured 3–60 mm (no severe local reactions [>210 mm] occurred). Low-grade fever occurred in 2.8 percent of the recipients.

CDC reports. As noted above, the botulinum toxoid is an Investigational New Drug and has not yet received FDA approval. As the holder of the IND, CDC submits annual progress reports to the FDA. These annual reports provide information on the number of injections given and summarize the adverse effects noted on the CDC Response to Investigational New Drug forms.[12] Additionally, the report provides summary statistics on injections since 1970. The committee examined five of the most recent progress reports (CDC, 1994b, 1995, 1996, 1997, 1998).

The CDC progress report for March 2, 1997, to March 1, 1998 (the most recent one reviewed by the committee) reported on 955 injections (728 primary and 227 booster injections) given to 422 individuals (CDC, 1998). Of the 955 total, 879 recipients (92 percent) noted no reaction or a mild reaction; 56 (5.9 percent) experienced a moderate reaction, 4 (0.4 percent) had a severe local reaction and/or systemic reaction,[13] and no response was recorded for 16 injections (1.7 percent). The four severe reactions were a 21-cm area of erythema and swelling, induration of approximately 35 cm, a severe local reaction, and severe swelling below the elbow.

Summary tables in the CDC report record the number of reactions for the 16,676 injections of the botulinum toxoid administered between 1970 and 1997 (CDC, 1998). Of this total, local reactions were characterized as 15,207 (91.2 percent) none or mild; 1,208 (7.2 percent) moderate; 63 (0.4 percent) severe; and no response recorded for 198 reactions (1.2 percent). Another summary table indi-

[12]The directions on the form state that it should be completed for each individual who receives the initial series and should be returned following the third injection. Additionally, a form should be completed and returned to CDC following each booster injection.

[13]Mild is defined as erythema, edema or induration 30 mm or less in any one diameter. Moderate is defined as edema or induration measuring greater than 30 mm but less than 210 mm in any one diameter. Severe is defined as any reaction measuring more than 210 mm in any one diameter or any reaction accompanied by marked limitation of motion of the arm or marked axillary node tenderness.

cated the number of systemic reactions from 1970 to 1997 (total of 747 systemic reactions): 348 reports of swelling, lumps, soreness, stiff back, or stiff neck; 87 reports of itching hives; 79 reports of general malaise; 69 reports of chills and fever; 63 reports of headache; 63 reports of nausea, diarrhea, vomiting, and GI problems; and 38 reports of blurred vision or dizziness. The report implies that all of these reactions were of limited duration.

Conclusions on Human Studies

Studies have noted transient local and systemic effects of the botulinum toxoid vaccine. However, studies of the botulinum toxoid vaccine have not used active surveillance to systematically evaluate long-term health outcomes. This situation is unfortunately typical for all but a few vaccines.

The committee concludes that there is sufficient evidence of an association between botulinum toxoid vaccination and transient acute local and systemic effects (e.g., redness, swelling, fever) typically associated with vaccination.

The committee concludes that there is inadequate/insufficient evidence to determine whether an association does or does not exist between botulinum toxoid vaccination and long-term adverse health effects.

The latter finding means that the evidence reviewed by the committee is of insufficient quality, consistency, or statistical power to permit a conclusion regarding the presence or absence of an association between the vaccine and a health outcome in humans.

MULTIPLE VACCINATIONS

Military personnel often receive several vaccinations as they prepare for service in an environment with many endemic diseases. People have been expressed concerns that multiple vaccinations prior to and during Gulf War service may have caused adverse health effects. In this section, the committee examines the issue of multiple vaccinations.

Vaccination programs in the U.S. military began in 1777 when Continental Army recruits received inoculation against smallpox (Takafuji and Russell, 1990). As vaccines were developed for other endemic diseases that would take a heavy toll on the health of the military population (e.g., diphtheria, yellow fever, influenza), vaccinations were made mandatory for U.S. troops.

Each of the military services determines the immunization requirements for its personnel. However, there is general consensus, as evidenced in the triservice regulation on immunizations (Table 7.1). Some vaccinations (e.g., measles–ru-

TABLE 7.1 Vaccinations Prescribed for Military Personnel

Disease or Agent	Army	Navy	Air Force	Marine Corps	Coast Guard
Adenovirus types 4 and 7	B	B	G	B	G
Vibrio cholerae	E	E	E	E	E
Hepatitis A	G	G	C,D	G	G
Hepatitis B	F,G	F,G	F,G	F,G	F,G
Influenza	A,B,X	A,B,R	A,B,R	A,B,R	B,C,G
Japanese Encephalitis	D	D	D	D	G
Measles	B,F	B,F	B,F	B,F	B,G
Meningococcus (types A, C, Y, W135)	B,D	B,D	B,D	B,D	B,G
Mumps	F,G	B,F,G	F,G	B,F,G	G
Polio	B,D,R	B,R	B,R	B,R	A
Plague	D,F	F	F	F	F
Rabies	F	F	F	F	G
Rubella	B,F	B,F	B,F	B,F	B
Tetanus-diphtheria	A,B,R	A,B,R	A,B,R	A,B,R	A,B
Typhoid	C,D	C,D	C,D	C,D	D
Varicella	F,G	F,G	F,G	G	F,G
Yellow fever	C,D	A,R	C,D	A,R	B,C,E

NOTE: A = All active-duty personnel; B = recruits; C = alert forces; D = when deploying or traveling to high-risk areas; E = only when required by host country for entry; F = high risk occupational groups; G = as directed by applicable surgeon general or Commandant, Coast Guard; R = reserve components; X = reserve component personnel on active duty for 30 days or more during the influenza season.

SOURCE: U.S. Department of the Air Force, 1995.

bella, influenza, diphtheria–tetanus) are administered routinely to all military recruits; others are administered for deployment to specific geographic or high-risk areas (e.g., typhoid, plague, cholera); and other vaccinations are specific to the occupational setting (e.g., hepatitis B vaccination for health care workers; rabies vaccination for animal control officers). During the first 2 weeks of military training, recruits may receive as many as 17 different antigens (Takafuji and Russell, 1990). Other vaccines may be given later in the training cycle or prior to deployment. The Armed Forces Epidemiologic Board, a group of civilian medical consultants, has been advising DoD since World War II on establishing and implementing DoD's preventive medicine guidelines. These guidelines are similar to civilian guidelines developed by the CDC and the Advisory Committee on Immunization Practices (IOM, 1996).

Combining several vaccines into a single injection or administering multiple vaccinations at the same clinical visit decreases the number of injections re-

quired to achieve protection against multiple diseases and reduces the number of visits to health care providers, thereby resulting in wider protection of the population (Goldenthal et al., 1995; Rappuoli, 1996; Choo and Finn, 1999). However, researchers continue to investigate whether interactions may decrease the efficacy of the component vaccines (i.e., as manifested in lower seroconversion rates and lower antibody titers) or increase the frequency of adverse effects.

In the United States, multiple vaccinations are routinely given to children, to adults prior to travel to areas of risk, and to military personnel. A number of combination vaccines have been licensed in the United States including: diphtheria–tetanus–pertussis (DTP), measles–mumps–rubella (MMR), 23-valent pneumococcal polysaccharide, and 4-valent meningococcal polysaccharide (Parkman, 1995; Ellis, 1996; CDC, 1999a; Choo and Finn, 1999). Additionally, safety and immunogenicity reports have appeared in the literature for several pediatric combinations including: DTP–hepatitis B (HBV); DTP–IPV (inactivated poliovirus vaccine)–HIB (*Haemophilus influenzae* type B); and MMR–varicella (Choo and Finn, 1999). Evidence suggests that although some combination vaccines interfere with immunogenicity, others enhance it (Insel, 1995). Short-term follow-up trials have shown these combination vaccines to be safe (Choo and Finn, 1999).

Some sets of vaccines can be administered simultaneously (i.e., on the same day but not at the same anatomical site) including yellow fever and measles; MMR and trivalent oral polio vaccine (OPV); and DTP, OPV, and MMR (CDC, 1994a; Insel, 1995; Parkman, 1995). In general, this practice does not appear to affect immune response to individual vaccines, nor does it result in substantial adverse effects in short-term (2–6 months and occasionally 2 years) follow-up studies (Grabenstein, 1990; CDC, 1994a; Parkman, 1995). The frequency of local and systemic reactions is generally the same as in separate vaccine administrations; however some patients experience greater local tenderness (King and Hadler, 1994). CDC states that simultaneous administration of inactivated vaccines that commonly produce local or systemic reactions, (e.g., cholera, parenteral typhoid, plague) may accentuate the reactions. CDC advises injections on separate days for these vaccines (CDC, 1994a). A study of the safety of the simultaneous administration of several childhood vaccines found that the proportion of MSAEFI (Monitoring System for Adverse Events Following Immunization, the precursor of VAERS) reports that described a set of specific adverse effects remained constant compared to the frequency reported for separate administrations, with one exception: local reactions were more frequent with simultaneous vaccinations (Chen et al., 1995). The MSAEFI system was a passive surveillance system based on reports by people who had been vaccinated, family members, and other individuals.

Toxicology

The intended effect of vaccination is to protect individuals from infection by stimulating the immune response to a particular antigen. Vaccination with

multiple antigens could possibly lead to polyclonal activation of the immune system. Webb (1997) has speculated that multiple vaccinations may shift the immune response to a Th2 profile that favors hypersensitivity responses. However, others have suggested that vaccinations may induce protective Th1 responses that should prevent the development of atopic disease (Barnes, 1999; Holt et al., 1999).

Animal Studies

Animal studies involving multiple vaccinations examine three categories of adverse effects: those specific to an individual vaccine (adverse health outcomes for anthrax and botulinum vaccines individually have been discussed earlier in this chapter), reduced protection to one antigen when the immune system is coping with multiple antigens simultaneously, and the development of adverse immunological reactions.

In examining animal studies on multiple vaccinations, the committee focused on studies in which at least one of the immunogens was the anthrax or botulinum vaccine. The committee did not examine the extensive literature on animal studies of vaccine combinations that have been in use for many years (use of the DTP vaccine began in the 1940s, and the MMR vaccine was approved in 1971; Plotkin and Mortimer, 1994).

Alteration in the protective effect of the vaccination. Multiple vaccinations may lead to an insufficient protective response to one or several of the antigens. Pilipenko and Miroshnichenko (1963) found that simultaneous injections of live vaccines for anthrax, plague, tularemia, and brucellosis in guinea pigs reduced the development of protective immunity to anthrax without adversely affecting the response to the other live vaccines. The only adverse effect of the vaccination itself was erythema and an infiltrate at the site of injection of the live spore anthrax vaccine, a response that resolved within 3 to 5 days. A similar study by Borodko and Samsonovich (1965) in guinea pigs reported a reduced immune response to all of the four antigens as determined by allergic skin responses and challenge with the infectious agent. The authors did not mention adverse effects from the vaccination.

In the following animal studies, the degree of protective immunity was unaffected by multiple vaccinations. In sheep, Safarov and Ibragimov (1968) investigated the combination of vaccination with live spore anthrax vaccine and the standard vaccine for braxy and infectious enterotoxemia. The studies did not mention any adverse effects from the vaccination itself. A study by Zuffa and colleagues (1972) combined vaccination with botulinum toxoid types C and D and vaccination for the viral Aujeszky's disease in mink; the authors did not discuss adverse effects. A similar study by Gorski and Motz (1984) found that vaccination with distemper virus at the same time as administration of botulinum type C toxoid did not affect immunity. The botulinum toxoid caused a small area of skin swelling that was evident for 2 weeks. Another study with

mink and ferrets (Shen et al., 1981) examined combined vaccination with live distemper virus, inactivated mink virus enteritis, and type C botulinum toxoid vaccine. Good protection was afforded to all antigens, and the authors did not mention any adverse effects. In guinea pigs, a comparative study was done to assess the efficacy of a protective antigen anthrax vaccine, either alone or in combination with a pertussis vaccine (Turnbull, 1990). The authors did not mention adverse effects from administration of either vaccine. Studies by Ramyar and Baharsefat (1969) in sheep indicated that the administration of the anthrax live spore vaccine in combination with sheep pox virus with saponin resulted in local skin reactions. Adverse effects occurred at the same rate for animals vaccinated with both anthrax and pox virus compared to each vaccination alone.

Adverse immunological reactions. *Repeated exposure to the same antigen (Hyperimmunization).* Studies in experimental animals have shown that repeated or large-dose injection with a single antigen may result in adverse health effects in animals. These hyperimmunization studies have been conducted only in animals, and it is not known whether the much smaller doses of antigen normally administered to humans as vaccines would result in similar adverse health effects.

Many studies, including those of Germuth (1953), have demonstrated that intravenous injection of a single high-dose antigen (0.5 g of bovine serum albumin) will stimulate the immune system, antibody production, and the development of a hypersensitivity serum sickness-like response in rabbits, characterized by glomerulonephritis and arteritis. Repeated injection of rabbits with egg albumin (20–40 mg intravenously divided into approximately 20 vaccinations over 2 months) led to the development of antibodies to antibodies (Aho and Wager, 1961). In addition, extensive injections of rabbits with casein (0.5 g injected subcutaneously twice a week for up to 15 months or more) led to the development of amyloidosis (Giles and Calkins, 1958). However, amyloidosis can also occur in the absence of extensive vaccination (Anderson, 1971). Other studies have demonstrated that injection of Balb/c mice with mineral oil can lead to the development of myeloma (Azar, 1968; Potter, 1971). For unknown reasons, the development of myeloma in response to mineral oil is unique to the Balb/c mouse strain, strongly suggesting a role for the genetic composition of this mouse strain in this unusual response.

Exposure to multiple antigens. A multiple vaccination study by Classen (1996) examined adverse immunological reactions. Classen found that vaccination, whether with one antigen or many, may predispose certain laboratory animals to the development of autoimmune conditions. Classen used strains of rats and mice that spontaneously develop diabetes. These animals are models of human insulin-dependent diabetes mellitus, an autoimmune disease. NOD/MrkTacfBR mice were used as well as diabetic-prone and diabetic-resistant BB/Wor rats. Classen also used a group of MRL/lpr mice that develop an auto-

immune disease that closely resembles human systemic lupus erythematosus. Animals were immunized with a protective antigen-based anthrax vaccine, alone or with various combinations of the diphtheria–tetanus (DT) vaccine; combined diphtheria, tetanus, and whole-cell pertussis (DTP) vaccine; combined diphtheria, tetanus, and acellular pertussis (DTPa) vaccine; or plague vaccine. Researchers noted that the development of autoimmune disease in the groups of animals could be accelerated or inhibited depending on the timing of the vaccination. These experiments suggest that immune stimulation by vaccination may alter the development of autoimmune disorders. As expected, such alterations may depend on the genetic susceptibility of the animal to autoimmune disease.

Conclusions on Animal Studies

Studies in animals found no difference in the degree of protective immunity with multiple vaccinations. Studies have suggested that a single high dose of an antigen or repeated stimulation with the same antigen may result in the production of autoantibodies or malignancies. However, the antigen loads in these studies far exceeded those used in human vaccine schedules with repeated vaccination. One study (Classen, 1996) did suggest that vaccination, whether single or multiple, could influence the development of autoimmune disorders in genetically susceptible animals. Thus, although it is plausible that an exaggerated immune stimulation from vaccination could lead to long-term effects of autoimmune-type disease with the attendant multiorgan system pathology, no long-term animal studies supporting this hypothesis have been reported.

Human Studies

Studies of three populations are particularly relevant to examining the effects of intensive administration of a large number of vaccinations. The first population is a group of Finnish army recruits who received many routine vaccines during the first weeks of service. The second group of studies followed laboratory workers at Fort Detrick who received an intensive vaccination program for occupational reasons. Further, the committee examined several studies of Gulf War veterans.

Finnish Army Studies

A series of studies of Finnish military recruits who received many vaccinations investigated whether intensive vaccination would stimulate the immune system to develop autoantibodies (Aho et al., 1962, 1967). The autoantibodies measured in the studies were rheumatoid factor (RF) and immunoconglutinin (IC). All servicemen were healthy during the observation period. Blood samples were drawn before vaccination and on two instances afterwards. Table 7.2

TABLE 7.2 Vaccination and Testing Schedules

Day	Vaccinations	Blood Sample
Aho et al., 1962 (245 recruits)		
1	Salmonella, tetanus, mumps, polio	1st sample drawn
8	Diphtheria, smallpox	
22–26	—	2nd sample drawn
43–47	—	3rd sample drawn
Aho et al., 1967, Experiment A (189 recruits)		
1	Salmonella, tetanus, mumps, polio	1st sample drawn
15	Diphtheria, smallpox	2nd sample drawn
29	—	3rd sample drawn
Aho et al., 1967, Experiment B (192 recruits)		
1	Smallpox	1st sample drawn
15	Salmonella, tetanus, mumps, polio	2nd sample drawn
35	Diphtheria	3rd sample drawn

SOURCE: Adapted from Aho et al., 1967.

shows sample sizes and summarizes the vaccination and blood sample schedules. Study subjects appear to have participated in a single study.

Of the total of 626 individuals tested, sera from 13 individuals showed positive RF responses before vaccinations and remained so throughout (with no clear change in titer) (Aho et al., 1962, 1967). Among those that were negative before vaccinations, there were 6 transient RF reactions (testing positive in the second sample but negative in the third), 5 that were positive in both the second and the third samples, and 12 that were positive only in the final blood sample. The IC responses were studied in 189 of the servicemen (Aho et al., 1967). The IC titers increased rapidly and then declined. None of the 22 individuals with significant (greater than fourfold) immunoconglutinin titers had RF reactions. Immunoconglutinin responses occurred within 2 weeks, whereas RF responses appeared within 3–4 weeks. Study results suggest that transient RF and IC reactions may be related to the immune response to intensive vaccination. However, the number of subjects with positive results was small, and no control group was used to test for spontaneous fluctuations in RF response.

Fort Detrick Studies

A group of skilled laborers and laboratory workers employed at Fort Detrick, Maryland, was followed for up to 25 years to investigate the potential subclinical effects of intensive vaccination. These individuals had received numerous vaccinations because of their potential occupational exposure to a variety of virulent microorganisms. They were selected from the 700 employees because of their long and more intensive vaccination history. The study group

underwent clinical and laboratory examinations in 1956, 1962, and 1971 (Peeler et al., 1958, 1965; White et al., 1974). No members of the study group suffered unexplained clinical symptoms attributable to the vaccination program that required them to take sick leave.

1956 examination (Peeler et al., 1958). At the first examination in 1956, the ages of the 99 Caucasian male subjects ranged from 28 to 65 (with a mean of 40.1 years). All had been vaccinated against botulism, tularemia, Rocky Mountain spotted fever, Q fever, plague, typhus, psittacosis, and eastern, western, and Venezuelan equine encephalitis (referred to as the basic occupational vaccinations); 95 were also vaccinated against smallpox, 37 against brucellosis, 28 against anthrax, and 25 against diphtheria. The initial series of injections was followed by boosters every 6 to 12 months. The duration of the vaccination schedule varied between 8 and 13 years (with a mean of 10.4 years). In addition, these subjects underwent frequent skin tests with a number of antigens (average of 20 skin tests). On average, 2.8 reactions to the vaccinations were recorded per subject (with a range of 0 to 16).

These individuals were evaluated by a review of outpatient and hospital records, complete medical history ($n = 93$), physical examination at the time of the study ($n = 93$), and serum electrophoresis and an extensive array of laboratory tests ($n = 89$). The sera of 44 individuals (18–50 years) who had not undergone multiple vaccinations served as the control group for the electrophoretic studies (controls were not done for the other tests). The controls were not matched on age, gender, or occupational exposure.

The incidence of past illness in the study group was comparable to that of the general population for this age group. Unexplained clinical and laboratory findings at this exam included leukocytosis ($n = 20$), lymphocytosis ($n = 40$), and abnormalities in liver function tests ($n = 53$).

In the serum electrophoretic studies, the mean total serum nitrogen and albumin were significantly higher in the intensively vaccinated group. Also, there was poor separation of α_2- and β-globulin fractions in 23 of the subjects but normal separation in the control group. There appeared to be no relationship between the occurrence of this abnormal pattern and the subject's age, duration of immunization, number of skin tests, or administered antigen amount.

1962 examination (Peeler et al., 1965). In 1962, 76 members of the original study group were available for follow-up. The duration of immunization ranged from 12 to 18 years (with a mean of 15.3 years). In addition to the basic occupational vaccinations, all had been immunized against smallpox, 72 against anthrax, 70 against yellow fever, 66 against Rift Valley fever, 63 against tetanus, 54 against influenza, 37 against poliomyelitis, 34 against brucellosis, 20 against diphtheria, 1 against cholera, and 1 against typhoid–paratyphoid. The number of skin tests varied from 9 to 44 per subject (with an average of 30).

All study subjects were evaluated by a complete medical history, outpatient records, physical examination, serum electrophoresis (also performed in 1958),

and extensive laboratory tests. Sera of 64 individuals were also examined for γ-globulin level and were screened for rheumatoid factor. Seven of the subjects also underwent a gingival biopsy. Three men who had persistent proteinuria underwent a percutaneous renal punch biopsy for detection of amyloid; the results were normal. Sera were drawn from 102 healthy blood donors in the same age group to serve as a control group for the electrophoretic and hexosamine studies.

Again, none of the nonoccupational illnesses occurred at a higher rate than in the general population, and there were no clinical illnesses that could be attributed to intense vaccination. Clinical and laboratory findings that are unexplained by previous illnesses included leukocytosis (n = 11), lymphocytosis (n = 24, with 6 subjects having lymphocytosis at both examination times), and some abnormalities in tests of renal or liver function.

Serum electrophoresis results showed poor separation of α_2- and β-globulin factors (n = 19). The mean serum hexosamine level was significantly elevated in the intensively vaccinated group compared to the control group in both 1958 and 1962. Of the 64 sera exhibiting abnormalities on laboratory tests, 10 had γ-globulin levels above normal, 12 showed agglutination of anti-D coated cells, and 22 had positive latex tests for rheumatoid factors (3 subjects were positive on all tests). The amount of antigens received did not explain these positive results. In the seven individuals who did not receive vaccinations in the 2 years preceding this study, anti-γ-globulin factors could not be detected. The causes of the four deaths since 1956 (three acute coronary occlusions and one colon carcinoma) were deemed unrelated to the vaccination program. In the three postmortem examinations performed, there was no evidence of amyloid deposition or other abnormalities in sections of liver, spleen, and kidney.

1971 examination (White et al., 1974). In 1971, 77 members of the original study group were re-examined, ranging at that time from 43 to 79 years of age with a mean age of 55 years. In addition to the basic occupational vaccinations, at least 60 were vaccinated against Rift Valley fever, influenza, vaccinia, yellow fever, brucellosis, and anthrax; 41 against poliomyelitis; and 37 against diphtheria. The individuals had received an average of 55 skin tests (range = 6–93). The vaccination program was discontinued 10 months before this study.

As in the previous studies, each subject underwent a complete medical history, physical examination, electrocardiogram, and extensive laboratory tests. The control group consisted of 26 age-matched, long-term, civilian male employees of Fort Detrick who had never received special vaccinations and were not exposed to laboratory infections. The authors concluded that there were no clinical sequelae attributable to intense long-term immunization. Only one laboratory abnormality, elevated serum hexosamine, occurred that had also been described in the previous studies on the intensively immunized group. During the 1971 exam, there were slight but statistically significant differences in serum albumin, α_2-globulin, and β-globulin levels compared to the control group. By 1971, 11 of the original group had died; this mortality rate agrees with the rate

estimated from actuarial data. No amyloid deposition was found in the one biopsy and four postmortem examinations performed.

Summary and conclusions on the Fort Detrick studies. There were no clinical sequelae (e.g., neoplasms, amyloidosis, autoimmune diseases) attributable to intense long-term immunization in this well-studied cohort. None of the subjects suffered unexplained clinical symptoms requiring them to take sick leave that could be attributed to the vaccination program. There was some evidence of a chronic inflammatory response, as characterized by certain laboratory test abnormalities: elevated levels of hexosamine (an acute-phase reactant), slightly abnormal white cell counts, slightly abnormal liver function test results, and polyclonal elevations in levels of gamma globulins. However, these changes cannot necessarily be attributed to the vaccinations, since the workers studied were occupationally exposed to a number of virulent microbes.

This series of longitudinal clinical studies had several shortcomings. There was no comparison cohort and no attempt to make the employees in the study representative of the broader population of workers. Further, the outcomes may be due in part to the healthy-worker effect since the subjects were selected for the intensity and length of their vaccination history, and individuals who either left employment or discontinued the vaccination program were not considered. Thus, the studies may have inadvertently focused on the most resilient individuals. However, the Fort Detrick study is valuable because careful monitoring did not disclose any evidence of serious unexplained illness in a cohort that received a series of intense vaccination protocols over many years.

Gulf War Veterans

Several studies of Gulf War veterans have looked for associations between health outcomes and exposure to a variety of agents, including vaccinations. The methodology and general results of these studies are described in Chapter 2.

U.K. Gulf War Veterans. Unwin and colleagues (1999) reported the results of a large cross-sectional postal survey on a random sample of U.K. Gulf War, Gulf War era, and Bosnia conflict veterans. The Gulf War and Bosnia troops were vaccinated against hepatitis A and B, yellow fever, typhoid, poliomyelitis, cholera, and tetanus (routine vaccinations), as well as against biological warfare agents (plague, anthrax administered simultaneously with the pertussis vaccine). Of the Gulf War cohort (n = 3,284; response rate = 70.4 percent), 31.8 percent reported that they had their vaccination records. The study found no difference between veterans with and without vaccine records regarding age, education, health outcomes, or rank, except that those with records were more likely to be reservists. A substantial fraction, 61.9 percent, of Gulf War veterans reported symptoms of the multisymptom syndrome (characterized by fatigue, mood or cognition, or musculoskeletal symptoms) using the CDC criteria (Fukuda et al., 1998). The study found that having received any routine vaccination

was significantly associated with the multisymptom syndrome among all Gulf War veterans (odds ratio [OR] = 1.2, 95% confidence interval [CI] 1.1–1.4); however, when the analysis was restricted just to veterans who had formal records of their vaccinations, the association was not significant (OR = 1.0, 95% CI 0.7–1.3). There was also a significant association between reporting biological warfare vaccination and the multisymptom outcome in the Gulf War cohort (OR = 1.5, 95% CI 1.3–1.7). Data were available in the Bosnia cohort only for anthrax vaccination, where no significant association was seen (OR = 1.5, 95% CI 0.7–2.9). An association was seen for Gulf War veterans receiving the anthrax vaccine (OR = 1.5, 95% CI 1.3–1.7). Servicemen who recalled experiencing adverse effects immediately after vaccination were more likely to have current symptoms (Gulf War cohort: OR = 2.8, 95% CI 2.4–3.3; Bosnia cohort: OR = 2.2, 95% CI 1.6–3.1). Controlling for these perceived adverse effects immediately after vaccination weakened the association in the entire Gulf War cohort between vaccination and the long-term multisymptom outcome, except for tetanus vaccination (OR = 1.2, 95% CI 1.0–1.4). Controlling for immediate short-term adverse effects after vaccination in the statistical analysis allows an examination of a possible direct association between vaccination and long-term effects. However, this statistical procedure would tend to underestimate the association between vaccination and long-term effects if short-term adverse effects were really correlated with long-term adverse effects; such a correlation is biologically plausible.

The total number of vaccinations received bore a weak but significant relationship to the occurrence of the multisymptom outcome among all Gulf War veterans, even when the cohort was subdivided into groups according to whether the subject possessed his vaccination records or not. The association still existed after controlling for the receipt of biological warfare vaccines and for experiencing side effects after vaccination (although the addition of this independent variable weakened the association). Among Bosnia veterans, no association between the number of vaccinations and the occurrence of the multisymptom outcome was observed. Although recall bias may be the reason for the significant results for individual vaccines, this is unlikely to be the case when the overall number of vaccinations is considered. Thus, the U.K. Gulf War study provides some limited evidence of an association between multiple vaccinations and long-term multisymptom outcomes. The respondents were more likely to be older, to still be in service, and to have attended the Ministry of Defence's assessment program for Gulf War veterans with symptoms. This study was conducted through questionnaire and relied primarily on self-reports.

A recently released study (Hotopf et al., 2000) reported on a further analysis of the United Kingdom data. This study focused on the subcohort of U.K. Gulf War veterans who reported that they had copies of their vaccine records (n = 923; 28 percent of responders in the original survey). The analysis examined the vaccines received, the timing of vaccinations, and six health outcome measures (the CDC-defined multisymptom outcome, psychological distress, posttraumatic stress reaction, fatigue, health perception, and physical functioning). The ques-

tionnaire also queried whether the servicemen recalled being exposed to pesticides or had experienced traumatic events during the Gulf War. The authors noted that the scheduling of vaccinations prior to and during the Gulf War was such that routine vaccinations were more likely to be administered before deployment and biological warfare vaccines were more likely to be administered during deployment. All regression analyses controlled for the possible confounding effects of rank, age, branch of service, and education.

The study found that the number of vaccines received prior to deployment was associated with one of the health outcomes (posttraumatic stress reaction). This relationship did not occur when accounting for the number of reported stressors. The number of vaccinations received during deployment was associated with five of the health outcomes (all but posttraumatic stress reaction). Once all vaccinations were taken into account in the analysis, the only two vaccines that showed an association with the CDC multisymptom syndrome were tetanus (adjusted OR = 2.7, 95% CI 1.0–7.2) and cholera (adjusted OR = 2.9, 95% CI 1.0–7.9). However, few records included these vaccinations (3.8 and 3.1 percent, respectively). In an analysis not controlling for all vaccines received, anthrax (OR = 1.4, 95% CI 1.0–1.8) and pertussis vaccination (OR = 1.4, 95% CI 1.0–1.9) were also significant. When the authors further analyzed the data to see if confounders (e.g., number of vaccines received simultaneously, number of years in the military, length of deployment to the Gulf, whether side effects of vaccinations were reported) could account for the association, the association held true.

This study is consistent with the hypothesis that receiving multiple immunizations within a narrow window of time, during a period of presumed stress (deployment to a theater of war), could be associated with the development of multiple symptoms and impaired functional status. One theory for which no direct evidence has been obtained in humans is that such a combination of events could cause alterations in the immune system, in particular a shift in the Th1 to Th2 response (Rook and Zumla, 1997). However, this study was limited by its cross-sectional nature and the fact that it relied on vaccine records that had been retained by only 28 percent of the survey respondents. Other possible confounding factors could be considered, including the timing of the vaccines (servicemen who received multiple vaccines during deployment tended to have been sent to the Gulf earlier, and medical personnel tended to report more vaccines during that period). Despite its limitations, this study was quite large and carefully evaluated the possibility of both confounding and response bias—concluding that neither explained its result.

CDC study. As part of the CDC study (see Chapter 2), Fukuda and colleagues (1998) performed clinical evaluations on a group of Gulf War veterans (n = 158), all of whom volunteered for the evaluation and were a subset of the index unit of Pennsylvania Air Force National Guard (45 percent of this unit had been deployed to the Persian Gulf). Participants in the clinical study were classified as cases (sufferers of the chronic multisymptom condition, as described in

Chapter 2) or noncases, based on responses to the questionnaire. As part of an extensive laboratory evaluation, the authors tested serum samples for antibodies to type A botulinum toxin and the anthrax protective antigen or lethal factor (to screen for exposure to the vaccines or toxins). The study found that 10 of the 158 individuals had antibodies to the botulinum toxin and 14 to the anthrax protective antigen; however there were no differences in reactivity rates between cases and noncases.

The clinical evaluation part of this study examined a small sample of veterans from one unit deployed to the Gulf War theater and did not rely on a random sample. The issues of bias and lack of power must be considered in interpreting the results of this study.

Conclusions on Human Studies

Certain multiple vaccination regimens can lead to suboptimal antibody responses, but there is little evidence, largely because of a lack of active monitoring, of other adverse clinical or laboratory consequences beyond the transient local and systemic effects seen frequently with any vaccination.

No long-term, identifiable clinical sequelae attributable to intense long-term immunization occurred in the Fort Detrick cohort. There was some evidence of a chronic inflammatory response, but these changes cannot necessarily be attributed to the vaccinations, since the workers studied were occupationally exposed to a number of virulent microbes. This series of longitudinal clinical studies also had several shortcomings. However, the Fort Detrick study is valuable because careful monitoring did not disclose any evidence of serious unexplained illness in a cohort that received a series of intense vaccination protocols over many years.

The U.K. Gulf War studies provide some limited evidence of an association between multiple vaccinations and long-term multisymptom outcomes, particularly for vaccinations given during deployment (Unwin et al., 1999; Hotopf et al., 2000). There are some limitations and confounding factors in these studies, and further research is needed.

> *The committee concludes that there is inadequate/insufficient evidence to determine whether an association does or does not exist between multiple vaccinations and long-term adverse health effects.*

This finding means that the evidence reviewed by the committee is of insufficient quality, consistency, or statistical power to permit a conclusion regarding the presence or absence of an association between multiple vaccinations and health outcomes in humans.

SQUALENE

The committee was asked to examine the literature on potential health effects of squalene since it has been raised as an issue of concern to Gulf War veterans. A recent GAO (1999a) report found that at the time of the Gulf War, DoD had concerns about having a sufficient quantity of the anthrax vaccine and sufficient time to fully immunize the troops (GAO, 1999a). However, DoD has stated that it decided against the use of novel adjuvant formulations (e.g., formulations with squalene) because of lengthy FDA relicensure requirements (GAO, 1999a). The following section provides a brief overview of the animal and human studies that have been conducted and concludes with the committee's thoughts on directions for future research. The committee was not asked to draw conclusions on the strength of the evidence for an association between exposure to squalene and adverse health effects.

Squalene[14] is a polyunsaturated terpene hydrocarbon that is widely distributed in nature. It is found in human sebum (a skin surface lipid) and is a precursor in the synthesis of human cholesterol (Final Report, 1982; Kelly, 1999). Its name stems from its abundance in shark (*Squalus* spp.) liver oil,[15] from which it was first isolated (Liu et al., 1976). Squalene also is found in other fish oils, olive oil (0.7 percent), wheat germ oil, rice bran oil, and many other foods.

For more than 25 years, squalene has been used commercially as an emollient for topical application of more than 300 cosmetic formulations, including suntan preparations, bath oil, eye makeup, hair preparations, lipstick, baby powder, and skin care preparations. Cosmetic concentrations range from less than 0.1 to more than 50 percent. Squalene also is available as a dietary supplement; as a constituent of certain pharmaceuticals, including suppositories; and as a carrier of lipid-soluble drugs (Final Report, 1982; Kelly, 1999). As described below, squalene is being investigated for a number of potential medical purposes.

Dietary Intake, Absorption, Distribution, and Metabolism

In the 1970s, the average dietary intake of squalene in the United States was calculated at 24 mg per day (given a daily dietary intake of 2,000 calories) (Liu et al., 1976). Olive oil is particularly rich in squalene. Among Asians, shark liver oil supplements containing squalene are popular over-the-counter folk remedies (Asnis et al., 1993). The average total squalene exposure of humans from all routes of administration does not appear to have been studied. In case studies, excessive ingestion of squalene from dietary supplements has led to lipoid pneumonia (Asnis et al., 1993).

[14]Its chemical formula is 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene.

[15]Squalene concentrations in shark liver oil range between 50 and 80 percent (Liu et al., 1976).

Squalene is absorbed through several routes of administration, depending on the species (Final Report, 1982). In mice, squalene penetrates slowly and poorly through the skin at a rate of 0.12 nmol/cm^2 per minute (Final Report, 1982). Subcutaneous administration in rabbits leads to increases of stored squalene in liver, muscle, and skin (Final Report, 1982). Virtually all squalene administered orally to rats (96–100 percent) is unabsorbed (Albro and Thomas, 1970).

In humans, about 60 percent of dietary squalene is absorbed through the gastrointestinal tract, with the remainder excreted in feces (Strandberg et al., 1990). A significant fraction of absorbed squalene is converted into cholesterol. Squalene is distributed throughout human tissues, with greatest concentrations in skin and fat (Kelly, 1999). Squalene in human serum comes from endogenous cholesterol synthesis and from diet (Strandberg et al., 1990; Kelly, 1999). Peak serum levels are attained 9–12 hours after ingestion (Gylling and Miettinen, 1994).

Animal Studies

There are few published studies of squalene toxicity in animals or humans (Kelly, 1999). Kamimura and colleagues (1989) examined subacute toxicity in dogs after a single oral squalene dose of 1,200 mg/kg. Over the next 3 months, accumulation was noted in several tissues, especially liver, but there were no signs of toxicity based on testing of serum and liver function. In contrast to humans, who absorb 60 percent of ingested squalene, this study reported that dogs absorb a relatively small percentage and excrete most in feces (83 percent). Thus, the relevance of this study to humans is unclear.

Squalene's toxicity is considered low, with an oral LD_{50} (median lethal dose) in mice at greater than 50 ml/kg (Final Report, 1982). No toxic responses were noted after subcutaneous and intramuscular injections of 0.5 ml per 20g mouse (25 ml/kg) of squalane ($C_{30}H_{62}$), a saturated and more stable version of squalene.

In a neuropathology study, squalene was administered subcutaneously to 10 rats (and 5 control rats) at a dose of 20 g/kg body weight for 4 consecutive days (Gajkowska et al., 1999). The rats' peripheral and central nervous systems were examined via electron microscopy 7 or 30 days from initiation of the experiment. After 7 days, disturbances in the myelin sheath were observed; these disturbances were more pronounced at 30 days. There was some swelling of Schwann cells in the peripheral nervous system. Fibroblasts were activated and showed signs of increased collagen production. In the central nervous system, squalene triggered swelling of astrocytes in white matter and in the hippocampus, especially near blood vessels. Lipid droplets accumulated in myelin in both the central and the peripheral nervous systems.

The pertinence of this neuropathology study is difficult to gauge because the dose was extremely high and the report provided minimal information about the study's methodology (especially handling of controls). Additionally, it is not uncommon to detect occasional astrocytic or neuronal swelling or mitochondrial swelling in electron micrographs of normal tissue. Further, damage appeared to be localized, not global, targeting, for example, a single myelin fiber or axon.

Squalene has attracted the interest of arthritis researchers because of its ability to activate the immune system nonspecifically. It was one of the constituents used in the 1970s to create the first animal models of multiple sclerosis, known as experimental allergic encephalomyelitis (EAE) (Beck et al., 1976). Squalene is one of several adjuvants (such as incomplete Freund's adjuvant) found to induce arthritis in *susceptible* rat strains and has been used in the generation of animal models of arthritis (Whitehouse et al., 1974; Lorentzen, 1999). The effect is so pronounced that researchers have coined the term "squalene-induced arthritis." After a single intraarticular injection of 50 μL squalene into Lewis (Yoshino, 1996) and Dark Agouti rats (Yoshino and Yoshino, 1994), animals experienced moderate joint inflammation by day 6, followed by more severe chronic arthritis by day 21. The inflammation was marked by joint swelling and infiltration of $CD5^+$ and $\alpha\beta^+$ T cells. Similarly, intradermal injection of 200 μL squalene into Dark Agouti rats produced arthritis (Lorentzen, 1999). Although the mechanisms are not fully understood, the inflammation is blocked by agents that suppress T cells (Yoshino, 1996; Sverdrup et al., 1998). Animal studies do not report whether injection of squalene produces antisqualene antibodies.

In summary, there is limited published information about squalene toxicity. The human relevance of what has been published is unclear because of species differences in absorption. Squalene has been found to produce arthritis and neuropathology under select conditions in animals; the relevance to humans of these toxicity findings is uncertain.

Use of Squalene as a Vaccine Adjuvant

Squalene is currently being studied for a number of medical purposes including treatment of hypercholesterolemia (Chan et al., 1996); as an antidote to reduce the toxicity of accidentally ingested drugs (Kelly, 1999); and as an adjunctive therapy in cancer treatment to potentiate the cytotoxic activity of some chemotherapeutic agents (Kelly, 1999). The area of research that is of particular relevance to this chapter is the use of squalene as a vaccine adjuvant or as a component of a vaccine adjuvant.

The dose of an adjuvant is typically small (in the microgram range), and the route of administration is usually intramuscular. Squalene has been tested primarily as one component of the vaccine adjuvant MF59. MF59 is an oil-in-water microemulsion, consisting of squalene, polysorbate 80 (Tween 80, polyoxethylene sorbitan monooleate), and sorbitan trioleate (Graham et al., 1996). FDA has not yet approved any experimental vaccines with squalene-containing adjuvants.

The safety and efficacy of MF59 has been tested in a number of animal species with recombinant and natural antigens. Both short-term (approximately 2 weeks) and long-term (8 months) studies have been conducted and have detected some minor and transient changes in clinical laboratory parameters and histopathology (Ott et al., 1995). As described earlier in this chapter, a study by Ivins and colleagues (1995) on numerous combinations of adjuvants with the

purified anthrax protective antigen found adverse effects from one of the adjuvant combinations containing squalene; other adjuvants containing squalene did not elicit adverse reactions. Tests of an HIV candidate vaccine with MF59 found no embryotoxic or teratogenic effects in dogs or rabbits (Ott et al., 1995).

Clinical studies of MF59 and other squalene-containing adjuvants have been conducted with candidate malaria, HSV (herpes simplex virus), HIV (human immunodeficiency virus), and influenza vaccines (Ott et al., 1995; GAO, 1999a). Study populations for the clinical trials have included adults, elderly, and children and infants (Ott et al., 1995).

HIV Vaccine Trials

Keefer and colleagues (1996) investigated the safety and immunogenicity of a candidate HIV-1 vaccine in combination with MF59, with or without an additional immune modulator, MTP–PE (muramyl tripeptide linked covalently with dipalmitoyl phosphatidylethanolamine). Vaccination with the candidate vaccine Env 2-3 in MTP–PE/MF59 was associated with significant adverse effects; severe, though short-lived, systemic and/or local reactions occurred in 15 of 30 vaccinees. In contrast, Env 2-3 in MF59 without MTP–PE was relatively well tolerated; severe local and/or systemic reactions occurred in only 2 of 18 subjects. There were no severe reactions in the eight subjects that received MF59 alone.

Graham and colleagues (1996) evaluated the safety and immunogenicity of another candidate vaccine for HIV, the recombinant glycoprotein 120, formulated with MF59 with or without MTP–PE. Vaccines that contained MTP–PE caused a greater number of moderate or severe local and systemic reactions (of 16 participants, 4 had local reactions and 13 had systemic reactions) than did vaccine formulated with MF59 alone. Of 16 vaccinees, 7 had local reactions and 0 had systemic reactions.

The National Institute of Allergy and Infectious Diseases (NIAID)-sponsored AIDS Vaccine Evaluation Group examined safety data from 1,398 HIV-negative, healthy volunteers who were enrolled in 25 multicenter, randomized double-blind studies evaluating 11 HIV candidate vaccines (Keefer et al., 1996). The study examined the adverse effects of a number of adjuvants, including MF59 and MF59 formulated with the biological response modifier MTP–PE. MTP–PE was associated with moderate to severe local reactions as well as with self-limited severe systemic reactions that resolved within 2–3 days. The same vaccines in the MF59 emulsion alone were well tolerated (Keefer et al., 1996).

Influenza Vaccine Trials

The safety and efficacy of MF59 have been evaluated in pilot studies (Keitel et al., 1993) and clinical trials (Martin, 1997; Menegon et al., 1999; Minutello et al., 1999). A study by Martin (1997) assembled data from eight randomized controlled clinical trials over four influenza seasons; 984 elderly

volunteers (older than 65 years) received the adjuvanted vaccine, and 823 elderly volunteers received a conventional influenza vaccine. More than 20 percent of the volunteers who received the adjuvanted vaccine had local reactions. Myalgia was the only systemic effect to have been significantly more common in those receiving the vaccine with a squalene-containing adjuvant (3.9 percent) than those receiving the vaccine without the adjuvant (1.8 percent). All adverse events were recorded for 1 week after vaccination. Hospitalization and mortality were followed during the influenza season. The group receiving the adjuvanted vaccine had similar hospitalization rates and lower mortality than subjects receiving the conventional vaccine.

To date, clinical studies of the MF59 adjuvant that contains squalene have not shown any adverse health effects beyond transient acute effects.

Gulf War Issues

A recent study by Asa and colleagues (2000) reports on the development of an anti-squalene antibody assay to detect antibodies to squalene in the circulation. Blood samples from 144 Gulf War era veterans or military employees, 48 blood donors, 40 patients with systemic lupus erythematosus (SLE), 34 patients with silicone breast implants, and 30 patients with chronic fatigue syndrome (CFS) were studied for squalene antibodies. The study reports that a blinded test of serum samples found antibodies to squalene in more than 95 percent of 38 veterans deployed to the Gulf War who developed chronic illness symptoms; in all of the 6 veterans not deployed to the Gulf War who developed chronic illness symptoms; and in none of 12 veterans deployed to the Gulf War who were healthy. In an unblinded test, the study reported antibody reactivity to squalene in 5 percent of blood donors, 10 percent of patients with SLE, 10 percent of patients with silicone breast implants, and 15 percent of patients with CFS.

This study has several shortcomings. The subjects were self-selected, rather than being chosen at random from a larger sample, which can introduce substantial selection bias and does not allow inferences to the broader population of Gulf War veterans. Sample sizes were small, and the study may suffer from misclassification errors since the group of Gulf War veterans categorized as healthy (n = 12) was not devoid of individuals with serious symptoms (1 had fibromyalgia, 1 had thyroid disease, 3 had memory loss, and 4 had chronic fatigue). Further, the report provides inadequate evidence that the assay is able to accurately detect antibodies to squalene. Many of the methods used in the study are not described; as a result it is not possible to fully assess the study's methodology or to reproduce the assay. The study did not attempt to demonstrate that the substance giving the positive response in the assay was found in the immunoglobulin G (IgG) fraction of serum where antibodies are found. Further, the authors did not show that the assay was specific to squalene. To prove the specificity of the assay, the investigators would have had to show inhibition, in a dose–response manner, with squalene and no inhibition with other substances, as is seen in most reports of new enzyme-linked immunosorbent assays (ELISAs).

The committee does not regard this study as providing evidence that the investigators have successfully measured antibodies to squalene.

Future Research Directions Regarding Squalene

As squalene continues to be investigated for a number of clinical uses, ongoing toxicity studies will provide the additional information that is needed about its toxicity, both in animals and in humans. It will be important to examine the relevance of animal studies because of species differences in the absorption of squalene and the susceptibility of certain strains of animals to squalene's effects. In considering future research directions, the committee focused on squalene's potential use as a vaccine adjuvant. Research questions that remain to be addressed include the following:

- What types of immune responses does exogenous squalene evoke?
- Does the immune response differ with the route of administration or entry (i.e., oral, cutaneous, intramuscular)?
- How does the response vary according to the dose of squalene?
- Is the presence of antibodies to squalene abnormal, and if so, what is their functional significance?
- Could antibodies to squalene represent the consequences of, rather than the cause of, a pathological process?

CONCLUSIONS

The committee felt it would be helpful to the reader to restate the conclusions from this chapter. The conclusions listed below are identical to those made at the end of the respective sections of this chapter.

Anthrax Vaccine

There is a paucity of published peer-reviewed literature on the safety of the anthrax vaccine. The committee located only one randomized peer-reviewed study of the type of anthrax vaccine used in the United States (Brachman et al., 1962). However, the formulation of the vaccine used in that study differs somewhat from the vaccine given to Gulf War veterans (and currently in use). The Brachman study (and other early experimental studies) found transient local and systemic effects (primarily erythema, edema, induration) of the anthrax vaccine. There was no long-term monitoring for adverse outcomes. The committee did not compare the incidence of transient effects with other vaccines.

Studies of the anthrax vaccine have not used active surveillance to systematically evaluate long-term health outcomes. This situation is unfortunately typi-

cal for all but a few vaccines. The committee strongly encourages active monitoring to evaluate the long-term safety of the anthrax vaccine.

To date, published studies have reported no significant adverse effects of the vaccine, but the literature is limited to a few short-term studies. Reviewing the large body of results that have not yet been published may enable more definitive conclusions about the vaccine's safety. The committee strongly urges the investigators conducting studies on the safety of the anthrax vaccine to submit their results to peer-reviewed scientific journals for publication.

The committee's findings are best regarded as an early step in the complex process of understanding the vaccine's safety, which began with the vaccine's licensure in 1970 and the 1985 FDA advisory panel finding that categorized the anthrax vaccine as safe and effective. Active long-term monitoring of large populations will provide further information for documenting the relative safety of the anthrax vaccine.

The committee concludes that there is sufficient evidence of an association between anthrax vaccination and transient acute local and systemic effects (e.g., redness, swelling, fever) typically associated with vaccination.

The committee concludes that there is inadequate/insufficient evidence to determine whether an association does or does not exist between anthrax vaccination and long-term adverse health effects.

The latter finding means that the evidence reviewed by the committee is of insufficient quality, consistency, or statistical power to permit a conclusion regarding the presence or absence of an association between the vaccine and a health outcome in humans.

Botulinum Toxoid

Studies have noted transient local and systemic effects of the botulinum toxoid vaccine. However, studies of the botulinum toxoid vaccine have not used active surveillance to systematically evaluate long-term health outcomes. This situation is unfortunately typical for all but a few vaccines.

The committee concludes that there is sufficient evidence of an association between botulinum toxoid vaccination and transient acute local and systemic effects (e.g., redness, swelling, fever) typically associated with vaccination.

The committee concludes that there is inadequate/insufficient evidence to determine whether an association does or does not exist between botulinum toxoid vaccination and long-term adverse health effects.

The latter finding means that the evidence reviewed by the committee is of insufficient quality, consistency, or statistical power to permit a conclusion regarding the presence or absence of an association between the vaccine and a health outcome in humans.

Multiple Vaccinations

Certain multiple vaccination regimens can lead to suboptimal antibody responses, but there is little evidence, largely because of a lack of active monitoring, of other adverse clinical or laboratory consequences beyond the transient local and systemic effects seen frequently with any vaccination.

No long-term identifiable clinical sequelae attributable to intense long-term immunization occurred in the Fort Detrick cohort. There was some evidence of a chronic inflammatory response, but these changes cannot necessarily be attributed to the vaccinations, since the workers studied were occupationally exposed to a number of virulent microbes. This series of longitudinal clinical studies also had several shortcomings. However, the studies are valuable because careful monitoring did not disclose any evidence of serious unexplained illness in a cohort that received a series of intense vaccination protocols over many years.

The U.K. Gulf War studies provide some limited evidence of an association between multiple vaccinations and long-term multisymptom outcomes, particularly for vaccinations given during deployment (Unwin et al., 1999; Hotopf et al., 2000). There are some limitations and confounding factors in these studies, and further research is needed.

The committee concludes that there is inadequate/insufficient evidence to determine whether an association does or does not exist between multiple vaccinations and long-term adverse health effects.

This finding means that the evidence reviewed by the committee is of insufficient quality, consistency, or statistical power to permit a conclusion regarding the presence or absence of an association between multiple vaccinations and health outcomes in humans.

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8

Research Recommendations

The committee's charge was to review the scientific literature on the potential health effects of the agents to which Gulf War veterans may have been exposed. Of the many stressors and biological and chemical agents in the Gulf War theater, this report has reviewed the literature on the agents that were of most concern to the veterans and their representatives. Subsequent IOM studies will examine the literature on the remaining Gulf War-related agents.

The committee considered the evidence for each of the agents in turn, as if each one was the only risk factor for adverse health effects. It did so because the committee sought to learn how each agent, in the absence of all of the others, might affect human health. The committee realized through the course of this study, however, that there may also be a need to examine the impact of the total experience of deployment and war on veterans' health. Such an approach may help elucidate the nature of the illnesses in Gulf War veterans in a way that is not possible by examining single agents. Unfortunately, most of the studies conducted to date focus on single agents. Yet integrating the various stressors, biological and chemical exposures, the complexities faced by military personnel during all phases of deployment, and the issues surrounding war may provide a more realistic approach toward understanding veterans' health and may provide insights for preventing illnesses in future deployments.

The committee recommends research on the interactions among the multiple stressors, and biological and chemical agents to which military personnel were exposed as a result of the Gulf War conflict.

The committee has developed the following additional recommendations for research based on its review of the literature on each of the putative agents (Chapters 4–7). These recommendations highlight areas of scientific uncertainty and gaps in research.

DEPLETED URANIUM

While a group of veterans exist with depleted uranium in their tissues, the majority of veterans' exposure to DU is unknown. The committee urges the continuation and expansion of efforts to model potential exposures to DU in various military settings (e.g., inside and outside vehicles damaged by DU munitions, other areas potentially contaminated by the dispersion of DU particles). Such efforts may result in a quantitative assessment of Gulf War veterans' exposure to depleted uranium. Further, the committee urges publication of the results in the peer-reviewed literature so that the studies may receive broad review.

The committee recommends the following avenues of research to complement our current knowledge of the health effects of depleted uranium.

The committee recommends long-term follow-up of veterans exposed to depleted uranium, including the Baltimore cohort and other veterans potentially exposed to depleted uranium (e.g., those involved in cleanup operations or radiation control units).

Long-term follow-up of the cohort of veterans who have undergone evaluation at the Baltimore Veterans Administration (VA) Medical Center since 1993 will continue to improve our understanding of the health effects of exposure to depleted uranium. The committee recommends expanding the cohort of DU-exposed veterans and including control cohorts of non-DU-exposed Gulf War-deployed veterans in this study.

Additionally, controlled, long-term morbidity and mortality studies of additional cohorts of Gulf War veterans, particularly those that may have been exposed to DU through different routes of exposure (e.g., those involved in cleanup operations and in radiation control units), would further the knowledge on health effects related to DU exposure.

The committee recommends continued follow-up of the cohorts of uranium processing workers, particularly studies that will incorporate more sophisticated analyses of the data.

The majority of the evidence on the human health effects of exposure to uranium is from studies of workers in uranium processing mills and other facilities. These cohorts are a valuable information resource, as they are, in many cases, large groups that have been studied for many years. Additionally, researchers have analyzed data across several of these cohorts to enable comparisons between the cohorts and to increase the possibility of observing rare health outcomes. The

committee encourages studies that will provide continued long-term follow-up of uranium processing workers, particularly those that incorporate new methods of measuring dose and utilize sophisticated statistical analyses.

The committee recommends additional studies in experimental animals to investigate specific effects of depleted uranium.

Animal studies provide the opportunity to carefully study the effects of depleted uranium in isolation from other exposures. Controlled experimental conditions provide a contrast with studies of veterans or workers in industrial settings as both populations have concomitant exposures to many other potential hazards. Of particular importance are studies of cognitive function, neurophysiological responses, brain DU concentrations, and the transport kinetics of DU.

SARIN

The committee recommends the following avenues of research to augment our understanding of long-term effects of exposure to sarin.

The committee recommends careful long-term follow-up of populations exposed to sarin in the Matsumoto and Tokyo terrorist attacks.

The Matsumoto experience shows that direct exposure to sarin, particularly in intermediate to high doses, is associated with the acute cholinergic syndrome. Further, follow-up studies of Matsumoto demonstrate that significant chronic symptoms from sarin exposure persist and include visual disturbances, fatigue or asthenia, and headache. These chronic symptoms appear to be dose dependent. Follow-up studies, with well-defined control populations, will provide information related to possible long-term health effects.

The Tokyo sarin experience also confirms that intermediate doses of sarin leads to the acute cholinergic syndrome. Visual disturbances are frequent sequelae of acute exposure. Neurophysiological testing of a small group of asymptomatic people exposed to sarin shows chronic changes in visual and event-related evoked potentials and vestibulocerebellar function months after the acute syndrome had subsided. While these neurophysiological data are suggestive of subtle, persistent central nervous system (CNS) effects from sarin, long-term follow-up studies are required.

Of particular importance is a study that would include a group of individuals that presented symptoms of acute sarin poisoning at the time of exposure, as well as a group that was involved in the incidents but did not experience acute illness. These two groups, together with an unexposed matched control group, would provide important information on whether the long-term sequelae already reported occur in those who have been exposed to subsymptomatic levels of sarin. Studies should include neuropsychological testing, electroencephalogram (EEG), evoked potentials, and vestibular testing.

The committee recommends studies in experimental animals to investigate the long-term effects of acute, short-term sarin exposure at doses that do not cause overt cholinergic effects and cause only minimal acetylcholinesterase inhibition.

Massive acute doses of sarin, through inhibition of neuropathy target esterase (NTE), can induce delayed neurotoxicity in some, but not all, animal species. Lower doses over long periods may also exert this effect, but more research is needed to substantiate this finding. Long-term alterations in the EEG of nonhuman primates occur after sarin administration at high doses, as well as at doses that do not produce acute signs of toxicity. The clinical and long-term significance of these EEG changes is unclear. Studies should describe concomitant changes at the behavioral, electrophysiological, and biochemical levels. Studies should test the hypothesis that repeated exposure to sarin—alone and in combination with other organophosphate (OP) pesticides or nerve agents—at doses that do not cause overt cholinergic toxicity can produce delayed polyneuropathy.

The committee recommends research on genetic factors that may alter susceptibility to sarin toxicity.

The enzyme paraoxonase inactivates sarin by hydrolysis. The human PON1 gene has various polymorphisms. The corresponding genotypes determine the catalytic properties of an enzyme that hydrolyzes certain organophosphates at two different rates depending on which polymorphism is present. Studies have suggested a relationship between these genetic polymorphisms and neurological impairment in Gulf War veterans. However, because of their small size the findings require confirmation in a larger population. Studies on genetic polymorphisms, particularly of PON1, may elucidate the nature of human susceptibility to sarin toxicity.

PYRIDOSTIGMINE BROMIDE

The committee recommends studies on chemical interactions between pyridostigmine bromide (PB) and other agents, such as stress, and insecticides.

Studies have suggested the possibility that combinations of chemicals (particularly insecticides) and stress result in greater toxic consequences than when each of these exposures is present alone. Possible synergistic interactions of PB with chemicals and stressors (environmental and psychological) require replication and confirmation, particularly studies exploring the possibility of toxic interactions resulting from exposure to chemicals at low doses and over long periods of time. Such studies may also shed light on whether or not PB (which is unlikely to initiate organophosphate-induced delayed neuropathy [OPIDN]) may promote other chemically induced neuropathies.

The committee recommends research on genetic factors (e.g., genetic polymorphisms of butyrylcholinesterase [BuChE] and paraoxonase [PON1]) that may alter susceptibility to the effects of PB.

Differences in genetic susceptibility are proving important in many disease conditions and will likely emerge as a major determinant of neurotoxicity in humans. The observation that a small percentage of troops taking PB has more severe acute side-effects than the majority, which experienced little or no discomfort, suggests a spectrum of vulnerabilities in sensitive individuals, where genetic differences may contribute to their ability to absorb and metabolize PB. Polymorphisms in BuChE have long been recognized, but whether they play a role in determining the outcome of PB exposure is unknown. The potential relevance of genetic polymorphisms of paraoxonase to PB toxicity is also unclear since PB is not a substrate for these enzymes. However, the role of genetic variations influencing the toxicology of PB is an attractive target for research.

The committee recommends epidemiologic studies on the possible long-term health effects of PB.

As previously noted in this report, there has been little attention given to studying long-term health effects of PB. However, preliminary clinical findings suggesting effects of PB on cognitive function, cardiovascular disease, and visual function warrant future study.

Epidemiologic studies that observe long-term adverse effects in patients with myasthenia gravis might be a first step. Although patients with myasthenia gravis may provide an opportunity to study the long-term health effects of PB, the higher doses of PB prescribed to these patients and the characteristics of their disease may make it difficult to draw comparisons from myasthenics to a group of healthy individuals.

VACCINES

As noted throughout this report, medical record keeping for Gulf War veterans was extremely poor. It is difficult, if not impossible, to understand the effect of vaccinations without adequate records documenting individual vaccination histories. The committee urges the Department of Defense (DoD) to implement proper practices for medical record keeping and systematic collection of baseline and follow-up health data related to vaccination.

The committee is aware of efforts by the DoD to collect data on health outcomes of the recipients of the anthrax vaccination and to conduct a long-term study of this cohort. The DoD has an opportunity to properly monitor recipients of this vaccine and to provide invaluable information about potential long-term health effects. This research should be a priority for the DoD.

The committee recommends a long-term longitudinal study of participants in the Anthrax Vaccine Immunization Program.

The committee urges DoD to actively monitor the participants of this program and systematically collect and analyze data about symptoms, and functional and disease status. Further, the committee strongly urges investigators conducting studies on the safety of anthrax and botulinum toxoid vaccines to submit their results to peer-reviewed scientific journals for publication. The process of peer review by fellow professionals, one of the hallmarks of modern science, ensures high standards of quality and will enable broad review of results.

Finally, the committee hopes that the following recommendations will complement areas of ongoing vaccine research.

The committee recommends long-term, systematic research to examine potential adverse effects of anthrax and botulinum toxoid vaccination in multiple species and strains of animals.

To date, animal studies have focused largely on the efficacy of the anthrax and botulinum toxoid vaccines, rather than on possible adverse effects of vaccination. Future research should consider issues related to potential long-term adverse effects of anthrax and botulinum toxoid vaccines; potential health effects of the combinations of these and other vaccines routinely given to armed forces personnel; and potential health effects of combinations of these vaccines and pyridostigmine bromide, exposure to organophosphate compounds, and stress.

The committee recommends a careful study of current symptoms, functional status, and disease status in cohorts of Gulf War veterans and Gulf War era veterans for whom vaccination records exist.

Although vaccination records are not available for most Gulf War veterans, cohorts might be identified for whom such records exist. It would be important to assemble several groups to study long-term adverse health outcomes of anthrax and botulinum toxoid vaccines; these cohorts should include: nonimmunized, deployed and nondeployed Gulf War veterans; and immunized, deployed and nondeployed Gulf War veterans.

This report takes its place alongside several other recent IOM reports on the health of Gulf War veterans. Although the conclusions and recommendations presented here will not end the controversy surrounding Gulf War veterans' illnesses, this report will provide a scientific basis for consideration by the Department of Veterans Affairs as they develop a compensation program for veterans. The committee hopes that its deliberations, along with the work of many others, will add to the body of accumulating knowledge about the health of Gulf War veterans.

Appendixes

A

Scientific Workshop Agenda

INSTITUTE OF MEDICINE
NATIONAL ACADEMY OF SCIENCES

Committee on Health Effects Associated with Exposures During the Gulf War

June 14–15, 1999
Cecil and Ida Green Building, Room 126
2001 Wisconsin Avenue N.W., Washington, D.C.

MONDAY, JUNE 14, 1999

8:15–8:30 a.m.	Opening Remarks Harold Sox, M.D. (*Chair*)
8:30–9:00 a.m.	War Syndromes and Their Evaluation Craig Hyams, M.D., M.P.H. *Naval Medical Research Center*
9:00–10:00 a.m.	Epidemiologic Studies on Gulf War Veterans
9:00–9:30 a.m.	The Iowa Persian Gulf Study Bradley Doebbeling, M.D. *University of Iowa College of Medicine*
9:30–10:00 a.m.	Health Study of Canadian Forces Personnel Lesbia F. Smith, M.D. *Department of Public Health Sciences,* *University of Toronto*
10:00–10:30 a.m.	Health Effects of Depleted Uranium David McClain, Ph.D. *Armed Forces Radiobiology Research Institute*
10:30–11:15 a.m.	Pentavalent Botulinum Toxoid: Human Clinical Safety—30 Years of Experience at a Biological Laboratory

	Anthrax Vaccine: Human Safety Data—25 years of Experience at USAMRIID and the Safety of a Recent Human Dose Reduction Study Phillip Pittman, M.D., M.P.H. *U.S. Army Medical Research Institute of Infectious Diseases*
11:15–11:45 p.m.	IOM study *Veterans and Agent Orange*, Methodology Andrew Olshan, Ph.D. *School of Public Health, University of North Carolina at Chapel Hill*
11:45–12:15 p.m.	Genetic Susceptibility James Hanson, M.D. *National Cancer Institute*
12:15–12:45 p.m.	Lunch
12:45–1:15 p.m.	Chemical Interactions: Pyridostigmine Bromide, DEET, and Permethrin Mohamed Abou-Donia, Ph.D. *Duke University Medical Center*
1:15–1:45 p.m.	PB and Growth Hormone Keith Friend, M.D. *M.D. Anderson Cancer Center*
1:45–2:30 p.m.	Biological Effects of Stress William Malarkey, M.D. *Ohio State University*
2:30–3:30 p.m.	Toxicant-Induced Loss of Tolerance Claudia Miller, M.D., M.S. *University of Texas Health Science Center at San Antonio*
3:30–4:00 p.m.	Chemical Interactions Ernest Hodgson, Ph.D. *North Carolina State University*
4:00–5:00 p.m.	Plenary Session
5:00 p.m.	Adjourn

TUESDAY, JUNE 15, 1999

1:30–2:00 p.m.	Exposure Assessment Issues Demetrios Moschandreas, Ph.D. (committee member) *Illinois Institute of Technology*
2:00–2:30 p.m.	Methodological Issues Charles Phelps, Ph.D. (committee member) *University of Rochester*
2:30–3:00 p.m.	Anthrax Vaccine Philip Brachman, M.D. *Rollins School of Public Health, Emory University*

B

Public Meeting Agendas

INSTITUTE OF MEDICINE
NATIONAL ACADEMY OF SCIENCES

Committee on Health Effects Associated with Exposures During the Gulf War

September 16–17, 1999
Cecil and Ida Green Building, Room 126,
2001 Wisconsin Avenue N.W., Washington, D.C.

THURSDAY, SEPTEMBER 16, 1999

9:00 a.m.	Welcome and Introductory Remarks Harold C. Sox, Jr., M.D. (*Chair*)
9:15a.m.–12:00 p.m.	Presentations and Committee Questions
9:15 a.m.	Lisa Spahr *American Legion*
9:30 a.m.	Pamela Kaires, M.D. *Gulf War Veteran*
9:45 a.m.	Tony Duff *Gulf Veterans' Association*
10:00 a.m.	SSG Edward J. Bryan *Gulf War Veteran*
10:15 a.m.	SSG V. Hammack *North Shore Veterans Counseling Service*
10:30 a.m.	Janyce E. Brown *Wife of Gulf War Veteran*

10:45 a.m.	Michael E. Naylon *Presidential Oversight Board for DoD Investigations of Gulf War Chemical and Biological Incidents*
11:00 a.m.	Edward S. Hyman, M.D. *Louisiana Medical Foundation*
11:15 a.m.	Mark Koepp, R.N. Tom Lane Harold Wilkey *Gulf War Veterans*
11:30 a.m.	Denise Nichols, R.N. *Gulf War Veteran*
11:45 p.m.	Dr. Doug Rokke *Jacksonville State University, Alabama*
12:00 p.m.	William Baumzweiger, M.D. *Cedars Sinai Medical Hospital, UCLA*
12:00–1:00 p.m.	Plenary Session: Comments and Questions from Audience, Presenters, Committee
1:00 p.m.	Adjourn

NAS Building, Lecture Room
2101 Constitution Avenue, N.W., Washington, D.C.

FRIDAY, SEPTEMBER 17, 1999

1:30–2:30 p.m.	Committee Discussion with Dr. Robert Haley
2:30–2:45 p.m.	Welcome and Introductory Remarks Harold C. Sox, Jr., M.D. (*Chair*)
2:45–4:15 p.m.	Presentations and Committee Questions
2:45–3:00 p.m.	Dan Fahey *Military Toxics Project*
3:00–3:15 p.m.	Fabian Moodie *Gulf War Veterans of Vermont*

3:15–3:30 p.m.	Meryl Nass, M.D. *Private Practice in Internal Medicine, Freeport, Maine*
3:30–3:45 p.m.	Victor Silvester *Operation Desert Shield/Desert Storm Association*

C
Methods of Identifying and Collecting the Literature

The primary task of this study was a review of the scientific and medical literature published from the 1940s to the present on the health effects of depleted uranium (DU), pyridostigmine bromide (PB), sarin, and the anthrax and botulinum toxoid vaccines. Early in the course of this study, methods to identify, collect, and disseminate the literature were discussed and decided upon.

ONLINE DATABASES AND OTHER SOURCES

The committee focused on a comprehensive search of relevant online databases to begin its literature review. Twenty-two online databases containing citations to literature covering biomedical, toxicological, chemical, and historical information were searched in March 1999 utilizing Dialog, a commercial database vendor. These bibliographic online databases (Table C.1) offer the most effective means of identifying international scientific literature and, in general, cover the time span from 1960 to the present. Although there is subject and content overlap, each database serves a unique function, has a distinct subject emphasis, and indexes literature not available elsewhere. Searches were customized to reflect the structure of each database. To be as comprehensive as possible, the searches were structured to retrieve all types of literature (e.g., epidemiologic studies, case reports, letters).

To maximize retrieval, the search strategy incorporated synonymous terms and, where appropriate, the Chemical Abstracts Service (CAS) Registry Number, which uniquely identifies individual chemicals. Terms were truncated in order to include their plural forms or alternate spellings. The searches also in-

corporated any relevant MeSH (Medical Subject Headings) terms or terms from the EMBASE thesaurus.

The search on pyridostigmine bromide used the term "pyridostigmine bromide" and its CAS registry number [101-26-8] to search any field of the bibliographic record. After eliminating duplicates, this search yielded 2,351 unique citations. A similar search was conducted for the terms "sarin," "cyclosarin," "cyclohexyl methylphosphonofluoridate," and the registry numbers [107-44-8] and [329-99-7] appearing in any field of the bibliographic record. This search yielded 1,634 unique items. Searches were also conducted to retrieve epidemiologic studies of populations exposed to organophosphate insecticides. The search for citations on the anthrax and botulinum toxoid vaccines used terms relating to the specific toxins and vaccines. This search yielded 1,490 unique items. Searches were also conducted to retrieve citations on multiple vaccinations and on squalene. The databases were searched for citations on uranium or depleted uranium using terms related to the element, occupational and environmental exposure, and health outcomes.

An additional search retrieved citations on studies of uranium miners and other occupations. The results from the two searches were combined and dupli-

TABLE C.1 Bibliographic Databases Searched

MEDLINE
Toxline
Cancerlit
HealthSTAR
CAB HEALTH
EMBASE
NTIS
PsycINFO
Enviroline
Environmental Bibliography
BIOSIS PREVIEWS
International Pharmaceutical Abstracts
Occupational Safety and Health
World Translations Index
Pascal
Life Sciences Collection
Conference Papers Index
Inside Conferences
Gale Group Health and Wellness Database
Dissertation Abstracts Online
SciSearch
OCLC's Epic database

NOTE: NTIS = National Technical Information Service; OCLC = Online Computer Library Center.

cates were removed, yielding a total of 4,044 citations. The committee also searched for literature on illnesses in Gulf War veterans and related concerns. In August 1999, the same search strategies were used to identify any citations on newly published literature pertaining to the committee's review. This update retrieved 274 citations, which were added to the committee's databases.

Since online databases were developed in the mid-1960s, few offer retrospective coverage. To identify pre-1960s literature, the committee staff examined volumes of *Index Medicus*. Additionally, staff and committee members examined reference lists of major review articles and books for relevant citations; extensive reference lists were provided by several sources including the *ATSDR Toxicological Profile on Uranium* and recent reports by RAND. The study also accessed the TOXNET factual databases available through the National Library of Medicine and the Cochrane Collaboration database. In addition to the above sources, input was received from veterans, interested persons, committee members, and speakers at committee meetings. The committee also examined more recently published studies that it identified.

LITERATURE COLLECTION, DISSEMINATION, AND ANALYSIS

All retrieved citations were entered into bibliographic databases, which at the end of the study contained 6,249 references to abstracts, journal articles, books, military and civilian reports, dissertations, and conference proceedings relating to the agents and to the Gulf War. Staff and committee members reviewed the citations and abstracts for relevance to the committee's task. Acquiring the full text of the published literature involved accessing the collections of the National Library of Medicine (NLM), the National Institutes of Health Library, the Himmelfarb Health Sciences Library of the George Washington University, and the National Research Council (NRC) Library, as well as use of the NRC's interlibrary loan service. Documents were also ordered from the National Technical Information Service and other sources. The committee's work greatly benefited from access to the NLM's Docline document delivery service. Citations and abstracts from foreign language journals were reviewed, and a number of the most relevant foreign language articles were translated into English.

The committee divided itself into four working groups (PB, DU, vaccines, sarin–cyclosarin) that were responsible for identifying, reviewing, and analyzing the relevant literature. The work of each of the working groups was also reviewed by committee members from other working groups, and was presented and discussed thoroughly by the entire committee at each stage of the writing and analysis.

D
Gulf War Illnesses and Recognizing New Diseases

Miriam Davis, Ph.D.[1]

Gulf War illnesses refer to a cluster of unexplained symptoms not recognized by the medical establishment as a new syndrome or disease (Chapter 2). This appendix provides information to illuminate the general process of how a new disease gains recognition. While not a committee product (see footnote), the IOM committee has reviewed this information and offers it to describe how medical organizations make decisions about new diseases, and what types of scientific evidence they marshal. It also points to social factors, including culture and economics, which weigh into decisions about new diseases.

HOW NEW DISEASES GAIN RECOGNITION IN MEDICINE

Medicine is teeming with examples of new diseases gaining recognition. Not merely a matter for historians, the emergence of a new disease carries contemporary significance. In the past two decades alone, acquired immunodeficiency syndrome (AIDS) and several other new infectious diseases have loomed large as global threats to public health. Their emergence has galvanized public health to reckon with the possible emergence of other new infectious diseases (IOM, 1992). Outside the province of infectious diseases, conditions such as posttraumatic stress disorder and eosinophilia myalgia syndrome have gained recognition. Those with less understood etiologies include chronic fatigue syn-

[1]Department of Epidemiology and Biostatistics, School of Public Health and Health Services, George Washington University, and independent medical writer.

drome, fibromyalgia, and irritable bowel syndrome, to name a few. Some of these conditions are, in fact, not genuinely "new:" they carry new labels for the same or a similar constellation of symptoms characterized, in some cases, up to a century ago.[2]

The recognition of a new disease is far from straightforward (Wegman et al., 1997). The simplest statement is that it is a *process* (Kety, 1974), often taking years. The purpose of the process is to demonstrate that patients are affected by a *unique clinical entity distinct from all other established clinical diagnoses*. The individual "steps" for gathering and interpreting evidence are not clear-cut. Evidence from biomedical research plays a prominent, but not necessarily exclusive, role in defining and classifying a new disease. Social factors, including culture and economics, influence the recognition, classification, and definition of a new disease (Rosenberg, 1988; Aronowitz, 1998; Wessely et al., 1998). The relative roles of scientific and social factors most likely vary from disease to disease, and era to era. The arbiters of what is a disease (and what is not) are health professionals, organized through public or professional organizations (Wegman et al., 1997), yet without explicit rules for decisionmaking, as explained later.

The process of disease recognition, in its most general features, is captured in Figure D.1. It must be understood at the outset that this is neither a linear nor a carefully articulated process. The process begins with detection of patients whose symptoms cannot be explained readily by existing diagnoses, hence the widely used term "unexplained" symptoms or illness. "Illness" is the term used to refer to patients' subjective experience of morbidity that they report to their clinicians, in contradistinction to what clinicians diagnose (Eisenberg, 1977). Clinicians or epidemiologists then look for patterns or clusters of symptoms that occur together in the same patient and across many patients. When patterns of symptoms are detected, experts formulate a working "case definition" that establishes classification criteria for a potentially new syndrome. A case definition typically contains a mix of clinical, laboratory, and/or epidemiologic criteria.

The development of the first case definition is a vital milestone designed to spur research and surveillance. More like a hypothesis than a conclusion, the first case definition is an early step in the process of identifying a new clinical entity. Case definitions are protean, frequently changing over time as new evidence comes to light. They are not designed for making diagnoses, which is considered the province of medical practice (Wharton et al., 1990).

A case definition seeks to formulate criteria that effectively identify and distinguish a new patient population from patient populations with recognized

[2]What is now diagnosed as chronic fatigue syndrome is strikingly similar to the nineteenth century descriptions of the condition labeled neurasthenia (Wessely et al., 1998). Lyme disease is an infectious disease that has been traced back to a disease characterized at the beginning of the twentieth century (Aronowitz, 1991).

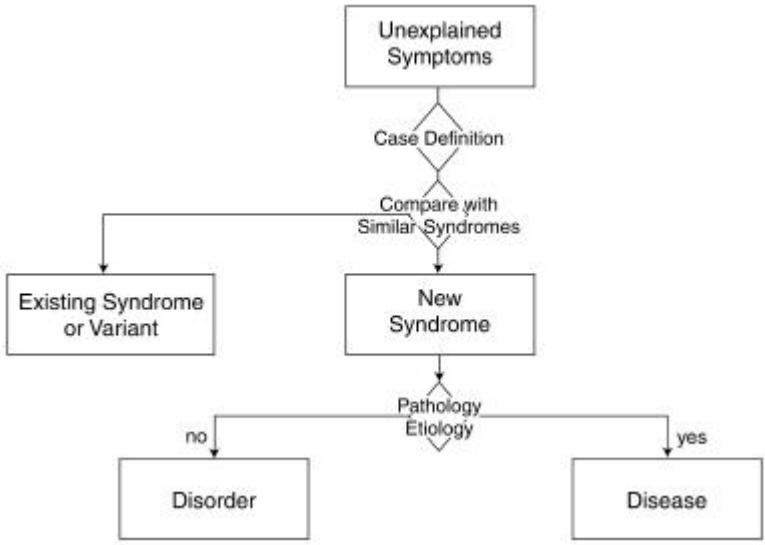

FIGURE D.1 General steps of disease recognition.

diagnoses that are often similar. Case definitions are usually the product of expert panels weighing the relevant body of research, which may include quantitative techniques such as factor analysis (Ismail et al., 1999). For example, several case definitions have been developed for Gulf War illnesses on the basis of factor analysis (Haley et al., 1997; Fukuda et al., 1998), yet none has gained strong acceptance, due either to methodological limitations or to lack of specificity (i.e., the inability to distinguish sufficiently between deployed versus nondeployed veterans). More refined case definitions are likely to emerge in light of ongoing research, and these are likely to elicit intense scrutiny. The discovery of a biological marker would likely prove decisive, as some researchers point out that a unique syndrome cannot be teased apart *solely* from veterans' symptoms (Ismail et al., 1999). The point is that existing knowledge of veterans' unexplained illnesses has not yielded a case definition that successfully specifies a new syndrome. That is why the prevailing medical convention is to resist the popular label "Gulf War syndrome."

When evidence is presented that a case definition is successful at singling out a new patient population from comparison groups, the case definition progresses a step forward: it begins to achieve recognition by the medical establishment as a new *syndrome*. The term "syndrome" is by convention reserved for a reproducible set or cluster of symptoms, signs, and/or laboratory tests, *without known pathology or etiology* (Scadding, 1996). The identification and labeling of a new syndrome is not, according to the medical model, an end in

itself. It too is an invocation for further research on etiology, pathology, course, and treatment (Kety, 1974).

As even more knowledge unfolds about etiology and pathology, an established syndrome can rise to the level of a disease. The term "disease" is often, but not always, reserved for abnormalities in body structure or function with known etiology (e.g., virus, abnormal gene, toxin, physical or psychosocial trauma) and/or pathology (detectable lesion). Diseases are considered mutually exclusive categories (WHO, 1992). Each disease is presumed to have a unique pathophysiology, but complete knowledge rarely exists (Scadding, 1996). According to the International Nomenclature of Diseases, the name of the disease should be specific, unambiguous, and self-descriptive and, if possible, should reflect the cause (WHO, 1992). In practice, the label acts as a shorthand description of the characteristic features of a disease that confer a biological disadvantage[3] and deviate from the norm (Scadding, 1996).

A syndrome can ascend the nosological hierarchy to a "disorder" even if there are *only* clinical manifestations (i.e., symptoms and/or signs) yet *no known* lesion. In contrast to a syndrome, the term "disorder" conveys that more is known about diagnostic reliability and validity, natural history, and impact on functioning (Goldman and Foreman, 1994). Impairment of functioning—social, educational, or occupational—is considered one of the quintessential criteria for a mental disorder. In its standard manual used for the classification and diagnosis of mental disorders in the United States, the American Psychiatric Association defines a mental disorder as a behavioral or psychological syndrome associated with distress and disability (i.e., impairment of functioning) (APA, 1994). Disorder is thus a more rigorous label than syndrome, but not as rigorous a label as disease, because the disorder's pathology or etiology are, by definition, not yet known. Yet there are always exceptions in deference to current or historical usage.

The advent of AIDS offers a recent illustration of how a new disease comes to be defined and labeled. The condition was entitled a "syndrome," according to an early case definition[4] promulgated by the Centers for Disease Control and Prevention (CDC) in 1982, before the cause was found. The syndrome was defined by the unusual combination of opportunistic infections and cancer (Kaposi's sarcoma) first detected in young male homosexuals (CDC, 1982). The rare combination of symptoms, signs, diagnoses, and young age bolstered the hypothesis that this was indeed a new syndrome. CDC's case definition for AIDS was modified several times within the first few years of the epidemic as research uncovered other populations affected (e.g., hemophiliacs), the mode of

[3]The "disadvantage" may also carry social components (Wakefield, 1992).

[4]The first unofficial label given to AIDS in 1981 was gay-related immunodeficiency syndrome or GRID (to signify the first known patient population), but this term disappeared as more cases appeared in different patient populations (Garrett, 1994). The first official case definition promulgated by CDC was "Kaposi's sarcoma and opportunistic infections in previously healthy persons" (CDC, 1982). Soon thereafter, the case definition was entitled AIDS (Garrett, 1994).

transmission, and the etiological agent (i.e., the human immunodeficiency virus) (Buehler et al., 1993). The most recent revision to the case definition was in 1993, a full 12 years after the first cases were recorded and 9 years after the cause was discovered. This attests to the protean nature of case definitions even, as in this example, with the advantage of objective physical findings and identified etiology. Eventually, the name of the condition was changed to "human immunodeficiency virus disease" to denote the etiological agent and to capture the full course of the disease, from primary infection, to asymptomatic stage, to late stage (WHO, 1992). Although AIDS is the term reserved for the late stage, nosologists would likely favor a name such as "late-stage HIV disease," but common usage reigns.

Systems of Disease Classification

Formal classifications of diseases, disorders, and syndromes are found in the latest modifications of the *International Classification of Diseases* (ICD-10) and the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV).[5] These two compendia are issued about every 10 years by the World Health Organization (WHO) and the American Psychiatric Association (APA), respectively. As the most authoritative sources for public health, government agencies, and health insurers, they have a myriad of applications for medicine, health statistics, reimbursement, disability claims, and medical record keeping. Numerous health and disability statutes at the federal and state levels require a code from one of these classification manuals. Thus, from a legal and statistical perspective, a new listing in these manuals marks the official "arrival" of a new clinical entity (Wegman et al., 1997), even though it may come as little surprise to many clinicians and researchers. From their point of view, a new listing is for coding and classification purposes, not necessarily for fundamental insights into etiology, diagnosis, and treatment. A listing can be construed as the reflection of consensus of health professionals, rather than the instigator.

The formal decision to place a new clinical entity in one of these volumes is made by health professionals organized under the auspices the sponsoring agency. WHO and APA have established procedures for making revisions to ICD-9 and DSM-IV, respectively (APA, 1994; ICD-9, 1999). Yet there are no explicit criteria underlying these procedures. Neither organization publishes explicit criteria for the types of research and clinical evidence needed to revise an existing listing or to add a new listing.[6] APA furnishes the most guidance about its procedures and the types of evidence that would warrant changes. It

[5]DSM-IV is a listing of mental disorders and ICD-10 is a listing of all somatic and mental disorders. The former enumerates specific criteria for making a diagnosis, whereas the latter does not. Its list of mental disorders is almost identical to those categorized by, yet expanded upon, in DSM-IV.

[6]WHO is presently engaged in the long-term process of developing an updating process between revisions that will include "an effective updating mechanism" (WHO, 1992).

convenes work groups to evaluate the body of clinical and research literature bearing on a potential revision and sometimes sponsors the reanalysis of existing data and the conduct of field trials. The types of evidence that APA considers important for a listing are clinical utility and diagnostic reliability and validity,[7] among other factors (APA, 1994). What is not specified is how this type of evidence is amassed and what its relative importance is for decisionmaking.

How to establish the validity of a syndrome or disorder is a formidable task when there are no biological measures of pathology or etiology (Faraone and Tsuang, 1994). Since this is a problem that disproportionately, but by no means exclusively, weighs upon mental disorders, mental health researchers are actively engaged in refining innovative methods to measure validity. Drawing on techniques developed by statisticians, mental health researchers seek to define clinical entities—in the absence of a diagnostic gold standard[8]—through several types of reliability and validity testing, the most fundamental of which is concept validity. Concept validity seeks to answer the question, Is there a "true," but unobservable, latent state of illness? To answer this question, Faraone and Tsuang (1994) argue for the utility of analytical procedures that are variants of latent class analysis. Latent class analysis is similar to factor analysis insofar as it attempts to identify latent (unobserved) variables from a series of correlations between observed variables. Faraone and Tsuang recommend broader use of latent class analysis, envisioning it as a supplement to other types of clinical information that help to establish the validity of a diagnosis—namely, course, outcome, response to treatment, familial aggregation, and biological measures (if available) (Robins and Guze, 1970; Kendell, 1989).

Factor analysis is widely used in the social sciences but has had more limited application in medical research. It attempts to infer the existence of something that cannot be observed directly or verified empirically. In the application of factor analysis to Gulf War illnesses, researchers strive to infer, and thereby "define," a potentially new Gulf War syndrome on the basis of the cooccurrence (or associations) of certain self-reported symptoms. However, other syndromal "definitions" may be equally consistent with the observed associations. A drawback of factor analysis is that the findings can become reified, or treated as if they are true states of nature (Gould, 1981), rather than, as in this case, a means of grouping symptoms that cluster together.

Much of the effort in classifying disorders in the mental health field is directed at confirming the validity of previously recognized disorders, not establishing the existence of new disorders (APA, 1994). Awareness of the lack of guidance for defining new conditions prompted Wegman and colleagues (1997) to

[7]Reliability refers to the extent to which a measurement procedure yields the same results on repeated trials (either at different time points or across different raters). There are many different types of validity, the most relevant being construct validity, discriminant validity, and convergent validity.

[8]Defined by Faraone and Tsuang (1994) as a method of evaluation predicated on a known disease pathophysiology (i.e., an excellent indicator of true disease status).

pose this provocative question as the title of a recent publication: "How Would We Know a Gulf War Syndrome If We Saw One?" The authors propose five steps for defining a new disease: (1) establish that the complex of symptoms and other findings are sufficiently different from recognized disease entities;[9] (2) ensure that the boundaries of the definition are not too narrow or too broad to exclude a common etiology; (3) ensure that the condition can be observed and confirmed by a broad range of clinicians drawn from different fields of medicine;[10] (4) attempt to include considerations of cause and effect, such as identifying common exposures, susceptibilities, or demographics; and (5) recognize the social, financial, and political pressures that promote or discourage acceptance of a new category. The authors proffer these criteria to encourage more systematic approaches to defining a new condition from Gulf War symptoms (Wegman et al., 1997).

Implications of Disease Classification

The classification of disease facilitates further study of a defined patient population. This is the very first step in the path to progress in diagnosis, treatment, and ultimately prevention. Classification also stimulates further understanding of etiology, risk factors, and natural history of the disease, all of which are critical for the public health goals of primary prevention or prevention of disease-related disability. Classification fosters communication among health professionals and patients, enhances surveillance, and supports patients' claims for reimbursement and disability.

The diagnosis of disease by clinicians, as noted earlier, is distinct from, but often dependent upon, classification criteria. Clinicians have the liberty to make diagnoses according to their clinical judgment in the practice of medicine. With the advent of a new disease, they generally rely on classification criteria promulgated by specialty organizations of medical professionals (e.g., the APA). In the absence of such criteria, clinicians still have the prerogative to make diagnoses, however unorthodox.

For patients, the diagnosis of a newly defined disease appears to carry both benefits and risks (Wegman et al., 1997; Wessely et al., 1998). Although empirical research is limited, the greatest benefits are patient acceptance of treatment and possible compensation for treatment and disability. Other benefits of diagnosis are patients' feelings of legitimation and feelings of greater mastery over their symptoms (Woodward et al., 1995). Some research suggests that patients are even likely to improve because of what they perceive as an explanation for their illness (Wessely et al., 1998). Finally, a diagnosis may dissuade patients from seeking alternative or complementary treatments of unproven value and possibly high expense. However, staving off patients' pursuit of alter-

[9]In psychometrics, this concept is referred to as discriminant validity.
[10]This is similar to the psychometric concept of interrater reliability.

natives may succeed only as long as their ongoing relationships with medical professionals continue to be satisfactory for them (Ax et al., 1997).

The diagnosis also may carry disadvantages for patients, especially if the condition is stigmatized. Stigma is most pronounced for conditions falling under the categories of mental disorders and addictive disorders (Link et al., 1999). Stigma refers to negative attitudes and beliefs that motivate others to stereotype patients as malingerers, blameworthy, weak, or flawed, and/or to avoid socializing and working with them. Stigma can trigger low self-esteem, isolation, and hopelessness in sufferers (Penn and Martin, 1998). While most of the research on stigma is devoted to severe mental disorders, there is evidence of stigma being perceived by chronic pain patients (Marbach et al., 1990) and those with fatiguing disorders (Wessely et al., 1998). Patients with multisystem organic complaints such as headache and fatigue are especially resistant to receiving a diagnosis of a mental disorder for a host of reasons—the fear of not being taken seriously, stigma, and concerns about lower financial reimbursement (Sparks et al., 1994; Sharpe, 1998; Wessely et al., 1998). Lastly, a diagnosis of a new, yet poorly understood, disorder possibly may result in affirmation of patients' expectations of disability (Sparks et al., 1994; Wessely et al., 1998; Barsky and Borus, 1999). These concerns figure prominently in physicians' reluctance to communicate with patients a diagnosis of an unexplained illness (Woodward et al., 1995).

GULF WAR ILLNESSES AND RELATED HEALTH CONDITIONS

There are unmistakable parallels between unexplained illnesses in Gulf War veterans and several other unexplained illnesses. The most striking similarity is in symptom presentation. The symptom cluster of headache, fatigue, cognitive dysfunction, and muscle and joint pain—along with lack of objective laboratory findings—also applies to several unexplained illnesses in civilian and military populations. Other similarities are poor functional status, disability, and apparently chronic course (Table D.1). The fundamental question propelling research is to determine whether unexplained illnesses in Gulf War veterans are variants of, or distinct from, similar conditions.

This section first describes medically unexplained illnesses and then presents the evidence for overlap between unexplained illnesses in Gulf War veterans and civilian populations. By closely examining resemblances between illnesses in Gulf War veterans and civilian populations, researchers hope to acquire insights into etiology and risk factors, prevention, course, and treatment. In fact, the knowledge gained with other populations already has generated experimental treatments for veterans. Several treatments found effective in civilians with unexplained illnesses are being tested in Gulf War veterans, as this section explains.

Medically Unexplained Illnesses

There are numerous illnesses with a symptom profile resembling that seen in Gulf War veterans. The broad category often used to label these syndromes is variously called "medically unexplained illnesses," "medically unexplained symptom syndromes," "functional somatic syndromes," "chronic multisystem illness," or "symptom-based conditions." These labels refer to conditions marked by somatic complaints unaccompanied by objective laboratory findings or established causation. For simplicity, the remainder of this appendix refers to these conditions as medically unexplained illnesses, but any one of these labels could apply. That the nomenclature is still so variable is but one indication of the uncertainty enveloping these conditions.

In ongoing research, the medically unexplained illnesses most frequently compared with illnesses in Gulf War veterans are fibromyalgia, chronic fatigue syndrome, and multiple chemical sensitivity (for summary descriptions, see Boxes D.1–D.3). All three are characterized by multisystem somatic complaints, usually pain, headache, and fatigue (Table D.1, Table D.2). For this reason, patients diagnosed with one of these conditions frequently meet case criteria for one or more of the others (Buchwald and Garrity, 1994; Slotkoff et al, 1997; Donnay and Ziem, 1999). Chronic fatigue syndrome and fibromyalgia have such overlapping presentations that the two conditions may possibly be different presentations of the same underlying condition (Buchwald and Garrity, 1994; Buchwald, 1996). On the other hand, a factor analysis study has offered evidence that chronic fatigue syndrome and fibromyalgia are distinct clinical entities (Robbins et al., 1997). The research question of whether the three conditions are separate conditions or variants of the same underlying condition is likely to remain unresolved until more is known about the etiology and pathogenesis[11] of each. For the present, they are considered discrete conditions, as reflected by separate case criteria and/or separate listings in ICD-10 (WHO, 1992; see Table D.1).

Other medically unexplained illnesses, documented in military populations from past conflicts tracing back to the U.S. Civil War, include DaCosta syndrome, effort syndrome, and combat stress reaction (Hyams et al., 1996). Several other medically unexplained illnesses, investigated mostly in civilian populations, have been labeled sick building syndrome, irritable bowel syndrome, and silicone-associated atypical rheumatic disease (Hyams, 1998; Wessely et al., 1999). The names of some of these conditions are often disputed, as is their very existence as distinct clinical entities (AMA, 1992; AAAAI, 1999). Some regard unexplained illnesses as manifestations of depression, anxiety, or somatization

[11]Pathogenesis refers to the series of biological events that give rise to the manifestation of symptoms or disease states.

TABLE D.1 Gulf War Illnesses and Related Conditions

	Unexplained Illnesses in Gulf War Veterans	Multiple Chemical Sensitivity (MCS)	Fibromyalgia (FB)	Chronic Fatigue Syndrome (CFS)
Most common symptoms	Fatigue Headache Muscle and joint pain Skin rash Impaired memory[a]	Fatigue, low energy Inability to concentrate Memory problems Nasal congestion Headache Throat soreness Joint discomfort[b]	Widespread muscle pain and stiffness Tenderness at specified soft tissues sites Fatigue Sleep disturbance Impaired cognition[c]	Severe fatigue Headaches Postexertional fatigue Impaired cognition Muscle pain Multijoint pain Sore throat Unrefreshing sleep Sudden onset of symptoms with a flulike illness[d]
Classification criteria	No widely accepted criteria[e]	No widely accepted criteria[f]	1990 American College of Rheumatology[g]	1988 CDC case definition, revised 1994[h]
ICD-10 listing	No	No	Yes[I]	Yes[j]
Causes	Unknown	Unknown	Unknown	Unknown
Pathology or laboratory test	None	None	None	None
Animal model	None	Neurobehavioral sensitization and/or limbic kindling[k]	None	None
Biological correlates	None yet identified	None yet identified[l]	HPA dysregulation Alterations in pain mediators (substance P, dynorphin) Growth hormone deficiency[m]	HPA dysregulation and other CNS abnormalities Immune activation Physical and cardiovascular de-conditioning[n]

Continued

TABLE D.1 *Continued*

	Unexplained Illnesses in Gulf War Veterans	Multiple Chemical Sensitivity (MCS)	Fibromyalgia (FB)	Chronic Fatigue Syndrome (CFS)
Patients with significant disability (%)	12% of Gulf War veterans receive disability from VA[u]	43% of MCS patients report disability[v]	26.5 % of FB patients report receiving disability payments[w]	Striking disability in role and social functioning and vitality 37% unemployed[x]

NOTE: CNS = central nervous system; NA = not available.
[a]Joseph, 1997; Murphy et al., 1999.
[b]More than 50% of 90 subjects reported these symptoms in an uncontrolled study (Ziem and McTamney, 1997).
[c]Wolfe et al., 1990; Buchwald and Garrity, 1994.
[d]Buchwald and Garrity, 1994.
[e]See Chapter 2.
[f]Research and clinical criteria are reported in Cullen (1987); Nethercott et al. (1993); and *Archives of Environmental Health* (1999).
[g]Wolfe et al., 1990.
[h]Holmes et al., 1988; Fukuda et al., 1994. Other criteria are used in the United Kingdom and Australia (Wessely et al., 1998).
[i]Listed as synonym under M79.0 "Rheumatism, Unspecified," under Diseases of the Musculoskeletal System and Connective Tissue (WHO, 1992).
[j]Listed under G93.3 "Post-Viral Fatigue" under Diseases of the Nervous System, as well as under F48.0 "Neurasthenia" under Mental and Behavioral Disorders (WHO, 1992).

[k]Bell et al., 1998a; Graveling et al., 1999. Sensitization is the progressive increase in the degree of response (usually locomotor or stereotyped movements in animals) over repeated presentations of a stimulus. Kindling, a subtype of sensitization, is the production of permanent susceptibility to seizures through repeated electrical or chemical stimulation of the brain at levels formerly incapable of triggering seizures.
[l]Simon et al., 1993.
[m]Russell, 1998; Bennett, 1998. HPA refers to hypothalamic–pituitary–adrenal axis.
[n]Bates et al., 1995; De Lorenzo et al., 1998; Demitrack and Crofford, 1998; Komaroff and Buchwald, 1998.
[o]Percentage with doctor-diagnosed "environmental illness" or "multiple chemical sensitivity" in population-based survey (Kreutzer et al., 1999).
[p]Wolfe et al., 1995.
[q]Buchwald et al., 1996; Similar prevalence found across different countries (United States, United Kingdom, Australia) using somewhat different criteria (Wessely et al., 1998).
[r]There is some evidence of effectiveness with cognitive behavioral therapy, but controlled clinical trials have not been performed (Sparks et al., 1994).
[s]Goldenberg, 1999.
[t]Sharpe et al., 1996; Goshorn, 1998; Komaroff and Buchwald 1998; Wearden et al., 1998.
[u]Cited in Hodgson and Kipen, 1999.
[v]Buchwald and Garrity, 1994.
[w]Wolfe et al., 1997.
[x]Bombardier and Buchwald, 1996; Buchwald et al. 1996.

BOX D.1
Chronic Fatigue Syndrome

Chronic fatigue syndrome (CFS), true to its name, is marked by severe and persistent fatigue, along with a cluster of other symptoms. Fatiguing syndromes, given names such as neurasthenia and DaCosta's syndrome, were chronicled 100 years ago and greeted thereafter with considerable dissent by the medical establishment (Straus, 1991; Wessely et al., 1998). The recognition and classification of CFS was transformed only in the past decade with the development of a case definition sponsored by the Centers for Disease Control and Prevention. The CDC's case definition, first published in 1988 and revised in 1994, requires fatigue, dysfunction, and four other defining symptoms at least 6 months' duration (Holmes et al., 1988; Fukuda et al., 1994). The latter symptoms most commonly include headaches, postexertional malaise, impaired cognition, and muscle pain (Buchwald and Garrity 1994). Established for research and surveillance purposes, the case definition also requires exclusion of several other disorders known to cause fatigue. Less than 1 percent of the population meets the case definition for CFS, although many more patients report chronic fatigue (Komaroff and Buchwald, 1998; Wessely et al., 1998). The etiology of CFS is unknown, and there are no accepted laboratory tests or pathological hallmarks (Epstein, 1995). Several biological correlates of the syndrome have emerged recently, including dysregulation of the hypothalamic–pituitary–adrenal axis, immune activation, and other measures (Goshorn, 1998). While infectious agents may trigger some cases of CFS, a complex, multifactoral etiology is proposed, incorporating biological, psychological, and social factors (Wessely et al., 1998). The degree of disability associated with chronic fatigue syndrome is striking, leaving high rates of unemployment (Bombardier and Buchwald, 1996; Buchwald et al., 1996).

(Hudson and Pope, 1989; Black et al., 1990).[12] These views are fueled by the well-documented coexistence of diagnosable mental disorders in a sizable subset of patients with several medically unexplained illnesses. However, many observers have pointed out the difficulty of disentangling cause and effect. Mental disorders such as depression and anxiety may be causes, risk factors, covariates, or consequences of medically unexplained illnesses (Abbey and Garfinkel, 1991; Hyams, 1998). Further, there is some evidence that patients with unexplained illnesses do not satisfy criteria for somatization (Buchwald and Garrity, 1994; Kipen and Fiedler, 1999).

Attempts to systematically study medically unexplained illnesses have been thwarted by problems in case definition and classification of patients (Hyams, 1998). The problems stem not only from the absence of abnormal physical signs

[12]Somatization refers to a group of mental disorders in which mental distress is expressed in terms of physical suffering (APA, 1994).

or laboratory tests, but also from the nature of the symptoms themselves. The symptoms are nonspecific and common, both in the community (Kroenke and Price, 1993) and in primary care (Kroenke et al., 1994). About 33 percent of patients in primary care, for instance, complain of four or more common symptoms (Kroenke et al., 1994). With nonspecific, common symptoms and no objective abnormalities, researchers have few guarantees that they are studying a homogeneous patient population. If the population is heterogeneous, this obscures researchers' ability to detect differences between those with the index condition and those without. This is referred to as the *problem of specificity*, and it plagues research on medically unexplained illnesses (Hyams, 1998). What is now grouped together as "unexplained illnesses" in Gulf War veterans, for example, might comprise heterogeneous illnesses with different etiologies, pathogenesis, and risk factors.

One potential solution to the problem of specificity is to take a dimensional, rather than a categorical, approach to identifying cases (Wessely et al., 1999). These and other investigators argue that the symptom overlap across medically unexplained illnesses[13] is so great that the differences represent an artifact of medical specialization or semantics (Clauw and Chrousos, 1997; Wessely et al.,

BOX D.2
Fibromyalgia

The hallmarks of fibromyalgia are widespread muscle pain and tenderness upon palpation at numerous preestablished soft tissue sites on the body, according to classification criteria promulgated by the American College of Rheumatology (Wolfe et al., 1990). The formulation of criteria was a watershed event in the evolution of a condition that had been described for more than a century and given various labels, the most recent of which was fibrositis. Other common symptoms entail fatigue, sleep disturbance, morning stiffness, and cognitive impairment, but these are not sensitive and specific enough to use for classification (Wolfe et al., 1990). Early characterizations of the condition as an inflammation of muscle (hence the label fibrositis) have not been borne out through research (Goldenberg, 1999). There is no pathological or laboratory test with which to confirm the diagnosis. Nor is there any widely accepted etiology. Fibromyalgia's prevalence is about 2 percent of the population, making it one of the more common rheumatological disorders (Wolfe et al., 1995). Fibromyalgia is 10 times more common in females, and its occurrence increases with age (Wolfe et al., 1995). On the basis of longitudinal studies, the course is chronic, yet variable in intensity (Wolfe et al., 1997). Several types of treatment have been found to be effective in controlled trials, including the tricyclic antidepressant amitriptyline, alone or in combination with fluoxetine (Prozac), as well as cognitive behavioral therapy and exercise. Anti-inflammatory and analgesic medications are no more effective than placebo (Goldenberg, 1999).

[13]Their lists of medically unexplained illnesses do not include illnesses in Gulf War veterans (Clauw and Chrousos, 1998; Wessely et al., 1999).

BOX D.3
Multiple Chemical Sensitivity

Multiple chemical sensitivity (MCS) is a controversial condition marked by heightened sensitivity to low levels of chemical exposures. Patients report disabling symptoms of fatigue, cognitive impairments, respiratory inflammation, headaches, among other symptoms, in uncontrolled studies (Ziem and McTamney, 1997). Although described by physicians since the 1950s, major medical associations have questioned the very existence of MCS (American College of Physicians, 1989; AMA, 1992; AAAAI, 1999). However, a recent evaluation of the biomedical literature, commissioned at the behest of the United Kingdom Health and Safety Executive, found "suggestive" evidence that MCS exists. Still, there are no pathological or laboratory tests. There are no widely used case criteria (Sparks et al., 1994). Most frequently, patients report that their symptoms are triggered or exacerbated by air pollution, cigarette smoke, solvent fumes, or perfumes (Buchwald and Garrity, 1994). No treatments for MCS have been studied in controlled clinical trials. On the basis of case studies and anecdotal reports, current treatments include avoidance (of the chemical[s] associated with symptoms), cognitive behavioral therapy, environmental control, diet, and sauna therapy (to mobilize and excrete toxins).

The etiology of MCS is unknown, although several models are being studied. The UK review cited above identified neuronal sensitization of the mesolimbic pathway of the brain as the etiological model with the best empirical support (Graveling et al.,1999). Sensitization refers to the progressive amplification of a given response after repeated exposures to the same stimulus. A battery of environmental chemicals (e.g., formaldehyde), endogenous substances (e.g., interleukin-2, corticotropin-releasing hormone), drugs (e.g., ethanol), and stressors (physical and psychosocial) can initiate neurobehavioral sensitization in animals (Bell et al., 1998a).

1999). A dimensional approach assumes that the defining features of unexplained illnesses (pain, fatigue, headache, etc.) occur as a continuum in the population, and that there are no distinct boundaries between people with different types of unexplained illnesses and those without them. Wessely and colleagues (1999) recommend a dimensional approach that divides patients with unexplained illnesses, not along categorical lines, but according to the number and chronicity of symptoms, associated mood disturbance, patients' attributions for symptoms, and identifiable physiological processes.

Evidence for Overlap

In past and ongoing research, Gulf War illnesses have been compared with fibromyalgia, chronic fatigue syndrome, and multiple chemical sensitivity (Boxes D.1–D.3). Fibromyalgia and chronic fatigue syndrome are more rigorously studied and more accepted diagnoses, as reflected by their inclusion

TABLE D.2 Overlap of Symptoms

	Fibromyalgia	Chronic Fatigue Syndrome	Multiple Chemical Sensitivity	Gulf War Illnesses
Symptoms:				
Back pain	X			
Joint pain	X	X	X	X
Extremity pain	X	X	X	X
Headache	X	X	X	X
Weakness	X	X	X	X
Fatigue	X	X	X	X
Sleep disturbance	X	X	X	
Difficulty concentrating	X	X	X	X
Nasal congestion			X	
Throat soreness			X	
Etiology	Unknown	Unknown	Unknown	Unknown
Pathology biomarkers	• HPA dysregulation • Growth hormone deficiency	• HPA dysregulation • Immune activation	Unknown	Unknown

in ICD-10 (WHO, 1992). Multiple chemical sensitivity (MCS) continues to be poorly characterized and more controversial among clinicians. One major reason is that chemical sensitivity at extremely low doses appears to defy principles of toxicology (Sparks et al., 1994; Reid, 1999). A recent review, commissioned by the United Kingdom Health and Safety Executive, identified "suggestive" evidence that MCS exists as a clinical entity (Graveling et al., 1999), yet this review is unlikely to quell skepticism about MCS.

The first indication of Gulf War veterans reporting symptoms consistent with these conditions emerged from the DoD and VA registries (Joseph, 1997; Murphy et al., 1999).[14] The large, well-designed epidemiologic studies summa-

[14]A diagnosis of fibromyalgia was made for 18 percent of 1,150 DoD registry patients referred for rheumatological evaluation of musculoskeletal complaints (Erickson et al., 1998). The overall frequency of fibromyalgia, chronic fatigue syndrome, and multiple chemical sensitivity in veterans participating in VA and DoD registries has not been published. Compilation would be difficult because in the early years, codes for chronic fatigue syndrome and fibromyalgia were not listed in ICD-9-CM, the coding system on which registries relied. Classification codes listing chronic fatigue syndrome (as "postviral fatigue syndrome" or "neurasthenia") and fibromyalgia were introduced in ICD-10 (WHO, 1992), but this classification system is not yet fully implemented in the United States. Neither ICD-9 nor ICD-10 contains codes for multiple chemical sensitivity. In

rized in Chapter 2 also furnished evidence of overlapping symptomatology. In the Iowa study, symptoms of fibromyalgia were reported by 19.2 percent of Gulf War veterans versus 9.6 percent of nondeployed controls. Symptoms of chronic fatigue were reported by 1.3 percent of veterans versus 0.3 percent of controls (Table 2.3).[15] Both findings were statistically significant (Iowa Persian Gulf Study Group, 1997). Similarly, in the study of Canadian forces sent to the Persian Gulf, symptoms of multiple chemical sensitivity, fibromyalgia, and chronic fatigue were significantly elevated in veterans relative to controls (Goss Gilroy, 1998). Finally, the study of U.K. veterans revealed symptoms of chronic fatigue syndrome to have been significantly heightened in Gulf War veterans in relation to Bosnia and Gulf era controls (3.3 percent in veterans versus 0.8 percent in both control groups) (Unwin et al., 1999). These three population-based studies were methodologically strong, based on either a random sample of the Gulf War veteran population (Iowa,[16] United Kingdom) or the entire Gulf War veteran population (Canada). Each study used veterans not deployed to the Persian Gulf for comparison purposes.

A questionnaire study of 1,935 veterans randomly sampled from the VA registry found 15.7 percent to qualify for chronic fatigue syndrome and 13.1 percent to qualify for multiple chemical sensitivity (Kipen et al., 1999). Smaller studies have focused on symptoms of chemical sensitivity in Gulf War veterans. In a pilot study of 48 veterans, Bell and colleagues (1998b) found that 12 out of 14 (86 percent) veterans reporting poorer global health status described themselves in a questionnaire as currently being chemically sensitive (in contrast to healthy Gulf War veterans and two other control groups of Gulf era veterans). Using a newly validated questionnaire to measure chemical sensitivity, Miller and Prihoda (1999) compared Gulf War veterans (n = 72), implant recipients (n = 89), individuals with multiple chemical sensitivity (n = 186) and healthy controls (n = 76). Chemical intolerance and symptom severity scores were significantly greater for all three groups than for controls.

The only published study thus far to have examined Gulf War veterans expressly for a *diagnosis* of multiple chemical sensitivity, as well as for chronic fatigue syndrome and fibromyalgia, was undertaken by Pollet and coworkers (1998). They performed physical examinations on 53 veterans who had volunteered for the VA registry with complaints of fatigue or chemical sensitivity. Of this group, 33 (62 percent) met diagnostic criteria for chronic fatigue syndrome, 20 for multiple chemical sensitivity, and 3 for fibromyalgia.[17] When compared

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March 1995, the VA registry program provided clinicians with separate codes (non-ICD codes) for chronic fatigue syndrome and fibromyalgia, but no diagnostic criteria were provided (IOM, 1998).

[15]This study did not examine the prevalence of symptoms of multiple chemical sensitivity.

[16]This study examined a random sample of all Gulf War veterans listing Iowa as the home of record at the time of enlistment (Iowa Persian Gulf Study Group, 1997).

[17]The figures are not additive because 14 had concurrent diagnoses of chronic fatigue syndrome and multiple chemical sensitivity, while others had no diagnosis.

with civilians diagnosed with chronic fatigue syndrome, the veterans in this group were judged to have a milder form of this condition on the basis of symptom severity, reduction in activity, and ability to work. Although this study did not compare the prevalence of these unexplained illnesses in deployed versus nondeployed veterans, it provides evidence that some veterans with fatigue and chemical sensitivity fulfill case definitions for chronic fatigue syndrome, multiple chemical sensitivity, and fibromyalgia. Further studies are in progress searching for similarities and differences between Gulf War illnesses and these related conditions (Research Working Group, 1999).

Benefits to Veterans

There are several immediate and potential advantages to veterans from investigating relationships between their illnesses and fibromyalgia, chronic fatigue syndrome, and multiple chemical sensitivity. These advantages may still hold even if research eventually demonstrates that Gulf War illnesses constitute a new clinical entity.

One benefit is that the body of research and clinical practice, especially for chronic fatigue syndrome and fibromyalgia, is more advanced than that for Gulf War illnesses. If even a subgroup of veterans with unexplained illnesses fulfill diagnostic criteria for any of these conditions, they form a more homogeneous population for study. The classification of patients, as discussed earlier, is a crucial first step, ushering in advances in treatment, etiology, and prevention, among other benefits. Another benefit is the possibility of studying long-term course. Since there have been no large *prospective* studies of Gulf War veterans from the time of their return, a key opportunity has been missed to elucidate the course of unexplained illnesses in this population (IOM, 1999). Prospective studies can be performed, however, in newly diagnosed civilian populations with chronic fatigue syndrome, fibromyalgia, and/or multiple chemical sensitivity. Such studies might indirectly benefit veterans by yielding novel insights about course of illness, determinants of severity, and approaches to prevention of disability.

Veterans also may benefit from treatments found effective in these other illnesses. This already has occurred with the initiation of a new treatment trial for veterans combining exercise and cognitive behavioral therapy (Engel et al., 1998), two mainstays of treatment for fibromyalgia and chronic fatigue syndrome (Table D.1). Cognitive behavioral therapy is a long-standing type of psychotherapy designed to alter faulty cognitions and replace them with thoughts and self-statements that promote adaptive behavior (Kazdin, 1996). This form of therapy seeks to replace self-defeatist expectations (e.g., "I'm going to be sick forever") with positive expectations (e.g., "I can get better"). Cognitive behavioral therapy also has been found effective in a randomized clinical trial for patients with many medically unexplained physical symptoms (Speckens et al., 1995).

Finally, new hypotheses about the etiology of Gulf War unexplained illnesses may be forged in light of findings from other unexplained illnesses. Their

etiology and pathogenesis are not well established, but there is no dearth of hypotheses guiding research. With fibromyalgia and chronic fatigue syndrome, several biological abnormalities have been detected in both sets of patients, including dysfunction of the HPA (hypothalamic–pituitary–adrenal) axis, leading to hypocortisolism (Demitrack and Crofford, 1998). Some observers postulate that the chain of causation begins with dysfunction of the central nervous system, which in turn triggers changes in immune function and changes in pain processing pathways (Clauw and Chrousos, 1997). Other biological correlates of chronic fatigue syndrome include anatomical abnormalities in subcortical white matter of the brain (via neuroimaging), chronic activation of the immune system, and reactivation of several latent viruses (Komaroff and Buchwald, 1998). With multiple chemical sensitivity, the etiology and pathogenesis are unclear. The model considered to have the strongest empirical support focuses on dysfunction of the mesolimbic pathway of the central nervous system (Graveling et al., 1999). Proposed by Bell and colleagues (1998a), the model posits that exposure to a host of exogenous and/or endogenous agents can elicit sensitization of neurons in the mesolimbic pathway. Because this pathway mediates autonomic, endocrine, and cognitive function, perturbations could lead to a broad array of seemingly unrelated symptoms. The model relates to chemical intolerance,[18] an unpleasant subjective sensation evoked by low-level exposures, rather than to multiple chemical sensitivity per se, which is a severe form of chemical intolerance. Since chemical intolerance also is common to chronic fatigue syndrome and fibromyalgia, Bell and colleagues (1998a) postulate that their model may also apply to these conditions.

SUMMARY

This appendix highlights the process of how a new disease comes to be recognized in medicine. The objective of this usually protracted process is to demonstrate through research that patients are affected by a unique clinical entity, one that is distinct from all other established clinical entities. The strength and coherence of research findings are not the only determinants of whether a new disease gains recognition by the medical establishment. Social factors, including culture and economics, also contribute to decisions, which are made by medical professionals. Professionals are convened under the auspices of the World Health Organization and the American Psychiatric Association, which publish their listings in the *International Classification of Diseases* and the *Diagnostic and Statistical Manual of Mental Disorders*, respectively.

[18]The advantage of studying chemical intolerance is that it can be measured in animal models. Other attributes of medically unexplained illnesses, such as fatigue, pain, and headache, are far more difficult to measure in animal behavior. Multiple chemical sensitivity is one of the few medically unexplained conditions for which an animal model has been developed (Table D.1).

Gulf War illnesses refer to a cluster of symptoms that remain unexplained. Thus far, there is insufficient evidence to classify veterans' symptoms as a new syndrome (Chapter 2). Still to be answered through more research is whether the symptoms do constitute a syndrome and, if so, whether the syndrome is genuinely new or is a variant form of other conditions, such as chronic fatigue syndrome, fibromyalgia, or multiple chemical sensitivity. This appendix has provided some evidence for overlap between Gulf War illnesses and these three conditions. New insights will be garnered from better understanding of the still-elusive etiology and pathogenesis of Gulf War illnesses.

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E
Effects of Long-Term Exposure to Organophosphate Pesticides in Humans

This appendix briefly reviews the epidemiological evidence for the long-term health effects of human exposure to organophosphates (OP) used as pesticides.[1] OP pesticide epidemiology has a bearing on the question of sarin toxicity for two primary reasons. First, the mechanism of action of OP pesticides and sarin is similar: they both bind to and inactivate acetylcholinesterase (AChE), thereby inducing elevations in the neurotransmitter acetylcholine (ACh) leading to an acute cholinergic syndrome. Their differences relate primarily to potency and duration of binding to AChE (Sidell and Borak, 1992). Second, OP pesticide exposures are much more common than sarin exposure, yielding greater evidence for examining potential health effects. There are about 10,000 cases of OP pesticide poisoning in the United States each year (cited in Steenland et al., 1994). This appendix addresses two questions related to OP poisoning:[2]

- What are the long-term health effects of an acute episode of OP poisoning?
- What are the long-term health effects of chronic low-level OP exposure (i.e., at levels insufficient to produce symptoms or signs of an acute cholinergic syndrome)?

[1]"Pesticides" is an umbrella term for any chemicals designed for pest control. They include insecticides, fungicides, and herbicides.

[2]An OP poisoning refers to having symptoms or signs of an acute cholinergic syndrome.

The committee restricted its review to major controlled epidemiologic studies that evaluated neurological, neuropsychological, and/or psychiatric symptoms and conditions in exposed versus unexposed individuals.[3] The committee excluded papers with only neurophysiological outcome measures, such as nerve conduction velocity or vibrotactile thresholds, because these appeared to be further removed from the symptoms typically reported by Gulf War veterans at the time of the committee's literature review. It was only later that Roland and colleagues (2000) reported on vestibular dysfunction in a small group of Gulf War veterans. This review is limited to publications after 1980, but earlier research supports the basic findings described here (Tabershaw and Cooper, 1966; Rodnitzky et al., 1975; Levin and Rodnitzky, 1976). A complete review of the health effects of OP pesticide exposure will be undertaken by the Institute of Medicine (IOM) in the second phase of this study.

Tables E.1 and E.2 summarize the major features of 15 published studies since 1980 that compare exposed and unexposed individuals to estimate the health effects of OP pesticide exposure. Table E.1 summarizes six studies that demonstrate the longer-term sequelae of OP pesticide poisoning. Table E.2 summarizes 10 studies, one of which is also included in Table E.1, that provide evidence of the longer-term health effects of chronic exposure to OP pesticides at levels insufficient to cause acute effects. Nearly all such studies are cross sectional, observational studies, which by nature are subject to common epidemiologic biases, including selection bias and reporting or information bias (see Chapter 3).

LONGER-TERM HEALTH EFFECTS OF ACUTE OP PESTICIDE POISONING

Table E.1 presents findings from six studies reporting on five distinct populations. All the studies were cross sectional, with neurological, neuropsychological, and/or psychiatric outcomes and with previous poisoning by an organophosphate as the main exposure variable. The time at which the health outcome was typically measured was *years* after the reported poisoning.

In four of the five populations, neuropsychological performance was significantly poorer in the group with previous poisoning (Savage et al., 1988; Rosenstock et al., 1991; Reidy and Bowler, 1992; London et al., 1998). In the fourth population (Steenland et al., 1994), the trend for neuropsychological performance was in the same direction as in the other four studies, but the difference did not achieve statistical significance. The fifth population was actually a subgroup of the fourth. In this subgroup, the poisoning was not sufficient to

[3] A similar yet more inclusive review of the health effects of organophosphate exposure has been conducted by the United Kingdom Department of Health Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (Woods, 1999). That report provides a more detailed review of 27 studies of the health effects of OP exposure.

cause hospitalization but was significant enough to depress blood cholinesterase levels by 60–70 percent. This study did not find consistent differences between exposed and unexposed populations (Ames et al., 1995). Three of the five populations exhibited increased rates of neurological or psychiatric symptoms in the previously poisoned group (Rosenstock et al., 1991; Reidy and Bowler, 1992; London et al., 1998).

Taken together, these cross sectional studies report a consistent tendency toward poorer neuropsychological performance and increased rates of neurological or psychiatric symptoms among persons with prior acute OP poisoning. The time from poisoning until evaluation in these studies is poorly documented but is typically on the order of years.

In each of these studies, analyses were conducted using exposure measured by previous acute exposure ("poisoning"). The poisonings were documented in most subjects on the basis of symptom reporting, hospitalization, and/or depressed cholinesterase levels. For example, the poisoned group studied by Rosenstock and colleagues (1991) had been hospitalized for OP poisoning and had no previous serious neurological or mental disorders. Steenland and colleagues (1994) obtained subjects from the State of California registry of OP poisoning. Given the severity of OP poisoning, there is likely to be little misclassification on this measure. The neuropsychological tests used in these investigations are varied, but are typically standardized and well defined, so that differential misclassification of the response is also unlikely.

Information bias is of greater concern in self-reported psychiatric and neurological symptoms. Persons previously poisoned who had suffered significant health consequences of OP pesticide exposure might be likely to report current symptoms differentially from persons not previously poisoned. The other major potential source of bias in these studies involves confounders that have not been adequately controlled for. Only one of the four studies (London et al., 1998) controlled for chronic OP exposures since the acute event. Hence, it is not possible to be sure that the longer-term sequelae of OP poisoning were due to the exposure that caused the original "poisoning" rather than to subsequent chronic exposures. London and colleagues (1998) did include prior poisoning and current job status as predictors of neurological symptoms and both were statistically significant—indicating that there may be health effects from chronic exposure (job status) over and above those from an acute exposure. There was little control for confounding in the study by Rosenstock and colleagues (1991) where OP exposure effects were most substantial. However, London and colleagues (1998) had the most detailed control for confounders and still found differences between exposed and unexposed groups.

In summary, the available literature indicates that exposure to OP pesticides at levels sufficient to cause acute health effects requiring medical reporting or treatment is associated with elevated rates of neurological or psychiatric symptoms and poorer performance on standardized neuropsychological tests several years after the acute exposure.

HEALTH EFFECTS OF CHRONIC LOW-LEVEL EXPOSURE TO OP PESTICIDES

Evidence on the health effects of chronic exposure to OP pesticides is presented in Table E.2, which lists results from 10 studies. Eight were cross sectional, one was longitudinal (Daniell et al., 1992), and another was a case-control study (Pickett et al., 1998). The cross sectional and longitudinal studies studied a total of 1,456 chronically exposed individuals and 817 controls. The case-control study compared the exposure to pesticides in 1,457 suicide cases to the exposure for 11,656 controls matched by age and province. One additional study by Burns and colleagues (1998) was not included in this review. Although this study presented important evidence on 496 workers at Dow Chemical who were chemically exposed to chlorpyrifos over a 17-year period compared to 911 workers who were not exposed, it was not possible to evaluate the findings because the duration of exposure, which differed substantially between the two groups, was not available from the paper, and apparently was not taken into account in the analysis. The study by London and colleagues (1998) is also included in the previous section.

The health outcomes in the eight cross sectional and one longitudinal study include neurological symptoms, neuropsychological tests, and psychiatric symptoms. The exposure variable was, in most cases, an indicator of whether the person's job involved exposure to OP pesticides or not. In cases where more detailed exposure information was available, it was most often not used in the formal analysis.

The only longitudinal study with repeated health assessments tested 57 applicators and 50 controls before and after a 6-month spraying season (Daniell et al., 1992). They found a pre–post change for only one neuropsychological (NP) test—the Symbol Digit test—which was significantly worse among applicators. Among the cross sectional studies, four conducted NP test batteries (Stephens et al., 1995; Fiedler et al., 1997; Gomes et al., 1998; London et al., 1998). Only Gomes and colleagues (1998) found poorer performance among exposed individuals on a substantial number of tests. This result was not replicated in the other three studies. These findings contrast with those studying people who were acutely poisoned (see previous section) where many NP test results were consistently poorer in the exposed group.

Six of the studies assessed neurological or psychiatric symptoms either by physician examination or by self-report. In five of the six studies, there was a statistically significant increase in the prevalence of symptoms. For example, Ciesielski and colleagues (1994) found increased likelihood of symptoms among persons with self-reported exposures. Stephens and colleagues (1995) reported increased vulnerability to psychiatric disorder as measured by the General Health Questionnaire. In the study of an Egyptian population, exposed workers had higher prevalence of depression, irritability, and erectile dysfunction (Amr et al., 1997). London and colleagues (1998) found that applicators were twice as likely as nonapplicators to have a higher overall neurological symptom score,

TABLE E.1 Human Studies of Organophosphate (OP) Pesticide Poisonings

Reference	Population		Health Outcomes	Exposure	Adjustment	Results
	Exposed	Control				
Rosenstock et al., 1991	35 hospitalized for OP poisoning	25 no prior OP poisoning	NP battery, psychiatric exam 1–3 years after hospitalization	OP poisoned or not	Matching: age	NP and psychological symptoms poorer in exposed group
	Population based; Leon, Nicaragua					
Reidy and Bowler, 1992	21 field workers with documented acute toxicity	11 cannery workers	NP battery; symptoms questionnaire	Acute toxicity or not	Matching: age, sex, education, SES	NP poorer for acute toxicity group
Steenland et al., 1994	128 men; OP poisoning California registry—same as Ames, 1995	90 male friends of exposed; no poisoning	Neurological tests; NP battery; Neurological exam 1–9 years after poisoning	OP poisoned or not	Regression: age, race, body mass index, language, alcohol, sleep, smoking, coffee, medications, current exposure	Trend only for NP poorer in exposed group

Savage et al., 1988	100 OP poisonings Colorado and Texas registries, 1950–1976	100 no poisoning	EEG and NP battery; neurological exam decades after poisoning	OP poisoned or not	Matching: age, gender, education, occupation, socioeconomic status (SES), race, ethnicity	NP and EEG tests poorer in exposed group; no differences in physical or neurological exams
Ames et al., 1995	45 men cholinesterase inhibited (CI) California—same as Steenland et al., 1994	90 friends not CI	See Steenland et al., 1994	Cholinesterase inhibited or not	See Steenland et al., 1994	No differences
London et al., 1998	164 pesticide applicators South African fruit farms	83 workers	NP battery, neurological symptoms, vibration sense, motor tremor	Prior poisoning, current applicator; long-term exposure by questionnaire	Regression: age, height, education, numeracy, visual acuity, alcohol, prior brain injury, current applicator	Neurological symptoms more prevalent among persons with prior poisoning controlling for current applicator status

TABLE E.2 Studies on Persons Not Previously Poisoned by Organophosphate (OP) Pesticides

Reference	Population		Health Outcomes	Exposure	Adjustment	Results
	Exposed	Controls				
Daniell et al., 1992	49 male pesticide applicators in orchards Washington State	40 slaughterhouse workers	Pre–Post NP battery	Pre–post measures over few months; applicator vs. control; cholinesterase levels	Preseason NP performance	Controlling for baseline NP performance, only symbol digit was worse in postseason for applicators
Ciesielski et al., 1994	202 farmworkers Two community health centers in North Carolina	42 nonfarmworkers	Self-reported symptoms over prior week	Self-reported pesticide exposure over prior month; erythrocyte cholinesterase levels	None	Only odds of diarrhea higher for subjects with low AChE; odds of many symptoms higher among persons with higher self-reported exposures including dysgeusia
Stephens et al., 1995	146 sheep farmers or dippers, no dipping in prior 2 months British Wool Marketing Board	143 quarry workers	NP battery, psychiatric symptoms (GHQ)	Sheep farmer vs. control; lifetime exposure by questionnaire	Regression: age, education, alcohol, comp. experience, language, time of day, smoking, accuracy	Sheep farmers worse on sustained attention and processing speed; greater vulnerability to psychiatric disorder on GHQ

Beach et al., 1996	20 sheep farmers, 10 highest symptoms; 10 lowest symptoms after dipping Population-based sample—British Wool Marketing Board	10 quarry workers	Neurological exam several months after dipping	Farmers with or without acute symptoms vs. quarry workers	None	Only two-point discrimination on hand–foot poorer among exposed farmers
Amr et al., 1997	208 workers at chemical (OP) manufacturing plant; 172 pesticide applicators	72 textile workers; 151 community controls	Psychiatric symptoms by GHQ	Chemical vs. textile workers	Matched by community, age, SES	Chemical workers or applicators had higher prevalence of depression, irritability, and erectile dysfunction
Fiedler et al., 1997	57 male tree fruit farmers; no poisoning New Jersey	42 berry farmers or hardware store workers	NP battery (NES); psychiatric assessment (MMPI-2)	Exposed group or not; lifetime exposure by questionnaire	None; regression: age, reading score	Only simple reaction time poorer in high-exposure group
Gomes et al., 1998	226 farmworkers in United Arab Emirates	226 nonagricultural workers	Neuropsychological tests	Farm vs. non farmworkers	Matched by age and nationality	Farmworkers poorer on aiming and digit symbol tests

Continued

TABLE E.2 *Continued*

Reference	Population: Exposed	Population: Control	Health Outcomes	Exposure	Adjustment	Results
London et al., 1998 (see also Table E.1)	See London above		NP battery, vibration sense, motor tremor, neurological symptoms	Applicator vs. control, lifetime exposure by questionnaire; plasma cholinesterase; occupation vs. nonoccupation	Regression: age, height, education, numeracy, visual acuity, alcohol	Applicators had 2.25 greater odds of high neurological symptoms controlling for past poisoning and other covariates; trend of more neurological findings among applicators
Pickett et al., 1998	Case-control study: 1,457 suicide cases vs. 11,656 controls		Suicide	Acres sprayed with herbicides, insecticides; costs of agricultural chemicals	Controls matched by age, province; logistic regression	No associations of suicide and insecticide or pesticide exposure
Azaroff and Neas, 1999	247 persons from 103 households Mestizo Indians—El Salvador		WHO symptom questionnaire and three dummy symptoms	Questionnaire of recent exposure (2 weeks, 1 year); urinalysis for alkylphosphate	Regression: age <18, gender, and their interaction	Several symptoms higher among OP+ who report exposure in last 2 weeks; several symptoms higher in persons living with a farmer who reported use of methylparathion in past 2 weeks

NOTE: EEG = electroencephalogram; GHQ = General Health Questionnaire; NES = Neurobehavioral Evaluation System; NP = neuropsychological; WHO = World Health Organization. OP+ = Individuals with a urinary test indicating detectable OP metabolites.

after controlling for past poisonings and other covariates. Finally, Azaroff and Neas (1999) reported increased rates of several symptoms among persons who had positive alkylphosphate, a biomarker of OP exposure, and a self-reported exposure in the past 2 weeks. Fiedler and colleagues (1997) performed a systematic psychiatric assessment using the Minnesota Multiphasic Personality Inventory-2 (MMPI-2), but did not find statistically significant differences between exposed fruit farmers and unexposed controls. In summary, there appear to be consistent patterns of increased symptom reporting among people whose jobs chronically expose them to OP pesticides but equivocal findings on standardized neuropsychological tests.

Pickett and colleagues (1998) conducted a case-control study comparing 1,457 Canadian farm operators who committed suicide over the period 1971–1987 to roughly eight times as many controls, matched for age and province. Their hypothesis was that exposure to pesticides was an important risk factor for suicide among farmers. This hypothesis was not supported because suicide cases did not have significantly increased past exposure, as measured by elevated acres sprayed with herbicide, acres sprayed with insecticide, or total expenditures on agricultural chemicals (after researchers controlled for a number of variables by logistic regression).

Taken together, these 10 studies provide mixed evidence about the association of standardized neuropsychological tests with chronic, subacute OP exposure. However, there are consistently higher prevalences of neurological and/or psychiatric symptoms, measured through either self-report or a standardized questionnaire such as the General Health Questionnaire, and no association with the occurrence of suicide.

Information, or reporting bias, is a serious consideration for this set of studies because persons who worked in jobs that exposed them to OP pesticides might differentially report symptoms thought or known to be associated with such exposures. The association with OP exposure was weakest for the suicide and standardized NP test outcomes, which are least subject to reporting bias. This association was greatest for the symptom data where reporting bias is more likely.

In summary, the extensive epidemiological evidence on the association of OP pesticide exposure and adverse health effects is consistent with and supports the more limited evidence on human exposure to sarin described in Chapter 5. There is consistent evidence that OP pesticide exposures sufficient to produce acute symptoms requiring medical care or reporting are associated with longer-term (1–10 years) increases in reports of neuropsychiatric symptoms and poorer performance on standardized neuropsychological tests. Workers exposed to OP pesticides at lower levels that did not produce acute effects also consistently reported higher rates of symptoms than controls but did not consistently perform poorer on objective NP tests.

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Acronyms and Abbreviations

ACh	acetylcholine
AChE	acetylcholinesterase
AFTAC	Air Force Technical Applications Center
AIDS	acquired immunodeficiency syndrome
ALP	alkaline phosphatase
APA	American Psychiatric Association
ATP	adenosine triphosphate
ATSDR	Agency for Toxic Substances and Disease Registry
A-V	atrioventricular
AVIP	Anthrax Vaccine Immunization Program
BBB	blood–brain barrier
BEIR	Committee on the Biological Effects of Ionizing Radiation
BMG	$beta_2$-microglobulin
BuChE	butyrylcholinesterase
CARC	chemical agent-resistant coating
CAS	Chemical Abstracts Service
CDC	Centers for Disease Control and Prevention
CEDR	Comprehensive Epidemiology Data Resource (DOE)
CFS	chronic fatigue syndrome

CHPPM	Center for Health Promotion and Preventive Medicine (U.S. Army)
CI	confidence interval
CIA	Central Intelligence Agency
CNS	central nervous system
CW	chemical warfare
DBS	Division of Biological Standards
DEET	*N*,*N*-diethyl-*m*-toluamide
DNA	deoxyribonucleic acid
DoD	Department of Defense
DOE	Department of Energy
DSM-III	*Diagnostic and Statistical Manual of Mental Disorders,* Third Edition
DTP	diphtheria–tetanus–pertussis
DU	depleted uranium
EAE	experimental allergic encephalomyelitis
EEG	electroencephalogram
EKG	electrocardiogram
EF	edema factor
ELISA	enzyme-linked immunosorbent assay
EMG	electromyography
ERP	event-related potential
FDA	Food and Drug Administration
FEV	forced expiratory volume
FFMPC	Fernald Feed Materials Production Center
FOIA	Freedom of Information Act
FR	fixed ratio
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
GAO	General Accounting Office
GC-MS	gas chromatograph–mass spectroscopy
gd	gestational day
GGT	gamma-glutamyltransferase
GH	growth hormone
GHRH	GH-releasing hormone
Hg	mercury
HI-6	1-[[[4-(aminocarbonyl)pyridinio]methoxy]methyl]-2-(hydroxyimino)methyl]-pyridinium dichloride monohydrate
HIAA	5-hydroxyindoleacetic acid

HIB	*Haemophilus influenzae* type B
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HPA	hypothalamic–pituitary–adrenal
HSV	herpes simplex virus
HT	5-hydroxytryptamine (serotonin)
IC	immunoconglutinin
ICD-9-CM	*International Classification of Diseases, Ninth Revision, Clinical Modification*
ICRP	International Commission on Radiological Protection
IDPN	iminodipropionitrile
Ig	immunoglobulin
i.m.	intramuscular
IMPA	isopropyl methylphosphonic acid
IND	Investigational New Drug
IOM	Institute of Medicine
IPV	inactivated poliovirus vaccine
IRS	Internal Revenue Service
i.v.	intravenous
LD_{50}	median lethal dose
LDH	lactate dehydrogenase
LF	lethal factor
LH	luteinizing hormone
MCS	multiple chemical sensitivity
MDPH	Michigan Department of Public Health
MeSH	Medical Subject Headings
MHC	major histocompatibility complex
MMPI	Minnesota Multiphasic Personality Inventory
MMR	measles–mumps–rubella
MPA	methylphosphonic acid
mRNA	messenger ribonucleic acid
MSAEFI	Monitoring System for Adverse Events Following Immunization
MTD	maximum tolerated dose
MTP	muramyl tripeptide
NAG	*N*-acetyl-beta-D-glucosaminidase
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health

NIOSH	National Institute for Occupational Safety and Health
NLM	National Library of Medicine
NP	neuropsychological
NRC	National Research Council
NTE	neuropathy target esterase
NTIS	National Technical Information Service
OCLC	Online Computer Library Center
OP	organophosphate
OPIDN	organophosphate-induced delayed neuropathy
OPV	oral polio vaccine
OR	odds ratio
OSAGWI	Office of the Special Assistant for Gulf War Illnesses (DoD)
P_{Emax}, P_{imax}	peak expiratory flow, peak inspiratory flow
PA	protective antigen
PAC	Presidential Advisory Committee
PAH	*p*-aminohippurate
2-PAM	praloxidime chloride
PB	pyridostigmine bromide
PD	pharmacodynamics
PEF	peak expiratory flow
PEP	primate equilibrium platform
PK	pharmacokinetics
PL	prolactin
POMC	pro-opiomelanocortin
PON1	paraoxonase/arylesterase 1
PPS	postpoliomyelitis syndrome
PTSD	posttraumatic stress disorder
PVR	peripheral vascular resistance
R_c	airway resistance
RBC	red blood cell
REM	rapid eye movement
RF	rheumatoid factor
RNA	ribonucleic acid
RNMCB	Reserve Naval Mobile Construction Battalion
RR	relative risk
SAM	surface-to-air missile
s.c.	subcutaneous

SCID-NP	Structured Clinical Interview
SF-36	Short Form-36 (Medical Outcome Study)
SLE	systemic lupus erythematosus
SMR	standardized mortality ratio
SSA	Social Security Administration
SWA	Southwest Asia
Ta	tantalum
TNF	tumor necrosis factor
TOCP	tri-*o*-cresyl phosphate
U	uranium
U.K.	United Kingdom
UNSCOM	United Nations Special Commission on Iraq
USAMRIID	U.S. Army Medical Research Institute of Infectious Diseases
U.S. NRC	U.S. Nuclear Regulatory Commission
VA	Department of Veterans Affairs (formerly, Veterans Administration)
VAERS	Vaccine Adverse Events Reporting System
VC	vital capacity
WHO	World Health Organization

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