CHEMICAL WARFARE AGENTS: TOXICITY AT LOW LEVELS

Satu M. Somani
and
James A. Romano, Jr.
Editors

© 2001 by CRC Press
Preface

We previously published a book on chemical warfare agents (Academic Press) in 1992. Since then, we have acquired considerable additional knowledge in this area. It is time to update our previous work, with particular emphasis on the low-level toxicology of chemical warfare (CW) agents. Chemical warfare agents are chemicals that have immediate, direct toxic effects on humans, animals, and plants and possible long-term, adverse effects on human health. Chlorine, phosgene, and mustard were CW agents used in World War I and in lesser conflicts thereafter. There was putative extensive use of CW agents in the Sino-Japanese War. Although CW agents were not used during World War II, much research was done in the development of toxicologic information and protective materials. However, mustard gas, defoliant, and nerve gases were used in localized wars in the 1960s, 1970s, and 1980s. Chemical warfare agents are primarily categorized as lethal and incapacitating agents. These agents also possess the attractive quality of being easy and inexpensive to synthesize on a large scale. A reasonable chemical-industrial set-up can be diverted to produce CW agents. Chemical warfare agents are particularly horrifying because their toxic effects are indiscriminate and thus affect not only military personnel but also the civilian population as a whole. Chemical warfare agents are becoming a major force in some of the militant developing countries. This is due to the fact that these agents can provide a substantial psychological edge to the military establishments of otherwise weak nations. Although acute toxicity and high-level dose toxicity were discussed in our previous volume, various review committees have suggested that there were data gaps in our information about the low-level toxicity of CW agents. The Gulf War of 1991 has raised our awareness of these gaps. Epidemiologic studies have indicated that more than 120,000 Gulf War veterans are suffering from many unexplained illnesses and are seeking medical care. Among the putative explanations for these illnesses include exposure to nerve agents or pretreatment drugs. Many United States and British troops were given pyridostigmine bromide as a pretreatment drug during 2 weeks of air and ground war to protect against the possible exposure to nerve gas. One of the notable nerve gases suspected to be present during the Gulf War was sarin. During war-time conditions, military personnel were under physical stress; some have argued for evidence of exposure to a low level of sarin. The toxicity of CW agents at low levels is a very special feature of this book. Certain factors such as stress, surroundings, and other chemical agents can interact with the toxicity of CW agents, and some of these interactions are described in this book.

There is a rapidly increasing interest in the low-level toxicology of CW agents. The National Institutes of Health, the Centers for Disease Control in Atlanta, the Veterans' Affairs Department, and the U.S. Army have a tremendous interest in this area, again stimulated by the aftermath of the Persian Gulf War. As a result of concern regarding a high incidence of undiagnosed illness among veterans of Operation Desert Shield/Storm, a Presidential Advisory Committee was formed to analyze the
full range of the Federal Government’s outreach, medical care, research, and coordinating activities pertinent to Gulf War Veterans’ Illness (GWI). The Presidential Advisory Committee also looked at short- and long-term health effects of selected Gulf War risk factors, e.g., chemical/biological (C/B) weapons, depleted uranium, infectious diseases, anti-biological warfare agent (BWA) vaccines, pyridostigmine bromide (PB), etc. The Presidential Advisory Committee gave specific and serious attention to the question of health effects of low-level exposure to nerve CW agents. To close this gap in the current knowledge base, the Department of Defense (DoD) was urged to support additional research on the long-term health effects of low-level exposures to CW agents (nerve agents in particular). Such an increased level of research has already been initiated, and elements of it are discussed thoroughly in various chapters.

The chapter contributors are experts well-recognized for their contributions to the science of toxic chemicals. Their contributions are summarized as follows:

Romano, McDonough, Sheridan, and Sidell provide an overview of the health effects of low-level exposure to nerve agents. They begin with description of the biochemical and physiologic actions of these agents leading to their toxicity. The authors describe the catastrophic effects of the use of these agents and the resultant previous emphasis on lifesaving therapeutic interventions. The authors then discuss reasons for the current emphasis on long-term health effects of these agents, particularly with respect to the question of “low-level exposure.” They attempt to provide workable definitions to the concepts of exposures and long-term health effects, review chronic health effects of acute exposures, review the contributions of in vitro studies to determine the health effects of low-level exposures and to provide a comprehensive, but perhaps not exhaustive, review of the literature surrounding chronic health effects of repeated low-level exposures, both animal and human. The authors close by expressing hope that the recent national investment into additional research will allow a more comprehensive assessment to unfold that will possibly contribute towards better treatment.

Benschop and DeJong provide a truly comprehensive review of the toxicokinetics of nerve agents. Their analysis includes toxicokinetics of G and V agents by inhalation or subcutaneous route, the influence of prophylaxis and therapy upon toxicokinetics of agents, and a chiral analysis of nerve agent stereoisomers. The development of this compendium of toxicologic data was partially dependent upon the development of improved methods of trace analysis in biological samples. Finally, the authors suggest that respiratory exposure for several hours to 20 ppb of nerve agent is near the lower limit of what can be reached with regard to toxicokinetics based on in vivo measurement of initial nerve agent. Further advances may enable reliable extrapolation of toxicokinetic results, even at low dosages, including extrapolation to man.

Somani and Husain described the low-dose toxicity of tabun, sarin, soman, and VX under normal as well as stressful conditions. These authors explained the interaction of environmental and physical stress on cholinergic as well as noncholinergic effects induced by low-dose exposure to nerve agents and their potential for additive or synergistic neuropathologic sequelae. Under certain conditions, nerve agents may
also induce delayed neurotoxicity called organophosphate-induced delayed neurotoxicity (OPIDN), which is characterized by inhibition of the enzyme, neuropathy target esterase or neurotoxic esterase (NTE). The clinical symptoms of OPIDN are muscular weakness of the hind limb and ataxia. This chapter deals with the delayed neurotoxicity in terms of behavioral, biochemical, and histological changes. The enzyme NTE can be used as a marker for assessing delayed neurotoxicity in humans or animals exposed to neuropathic nerve agents. Physical stress seems to potentiate the delayed neurotoxicity caused by low-dose exposure to sarin.

Soreq, Kaufer, Friedman, and Glick point out that the complexity of the blood-brain barrier (BBB) has hampered research efforts to delineate its components and fully understand its mode of action. However, there have been recent significant advances for evaluating BBB integrity. These new techniques include in vitro approaches such as cell culture, organ systems, and imaging approaches. In vivo approaches include ischemia resulting from, say, carotid artery occlusion or cold injury in mice. Finally, transgenic and knockout animal models have been developed, which are helping to elucidate critical factors in BBB integrity.

Sorani, Husain, and Jaganathan describe the pharmacokinetics and pharmacodynamics of carbamates (viz., pyridostigmine, physostigmine, or neostigmine) and several of the factors such as stress influencing them. Their extensive coverage of these compounds includes both human and animal studies. Among the potential uses for these compounds include their proposed use as pretreatments for nerve-agent poisoning by military personnel. Evidence supporting their effectiveness is presented and discussed. The pharmacokinetics of PB plays an important role in determining the pharmacodynamic effects in normal, disease, or stressful conditions, and in the presence of chemicals and low level nerve gas exposure. This chapter also discusses the pharmacokinetics and pharmacodynamics of physostigmine (PHY) under normal and stressful conditions. The influence of physical stress can at times be profound and these authors suggest that this area of research needs further exploration.

Doctor, Maxwell, Ashani, Saxena, and Gordon describe the progress made in exploring the use of enzymes to counteract the toxicity of organophosphorus (OP) compounds. They describe the use of cholinesterase scavenging enzymes, comparing these to a number of pharmacologic antidotes whose actions and efficacy are well known. These studies have involved several animal species. Special emphasis is placed on the use of HuBChE as a scavenging enzyme. Strategies to improve the bioscavenging capability of cholinesterases are described. These include amplification of effectiveness of ChE using oximes, site-specific mutagenesis of AChE, Huperazine A as a pretreatment drug, and the intriguing possibilities of immobilized cholinesterases to decontaminate and detoxify OP chemical warfare agents.

Lenz, Broomfield, Maxwell, and Cerasoli describe the use of scavenger enzymes as alternatives to conventional approaches to the management of nerve agent casualties. This new approach avoids side effects associated with current antidotal regimens. It also obviates the requirement, often difficult to achieve in a military setting, for rapid administration of pharmacologically sufficient drug to attain its therapeutic aim. Candidate bioscavenger proteins, which react quickly, specifically, and irreversibly with organophosphorus compounds are presented and discussed. This bond
may be stoichiometric and sequester substrate or may be catalytic, hydrolyzing substrate into biologically inert products. Promising examples of each approach are presented and the advantages of the novel approach over conventional approaches are discussed.

Hurst and Smith discuss the clinical effects that may arise from chronic, sometimes symptomatic, low-dose exposure. They make the argument that long-term health effects deriving from acute, subclinical asymptomatic injury do not occur. They discuss the appearance of chronic health effects following a period of chronic, subclinical exposure. They also discuss the possibility of a “threshold” for these effects by describing the outcomes of more than 30 years of use of a sulfur mustard-containing petroleum formulation to treat psoriasis. They duly note the extensive evidence of a carcinogenic effect after repeated occupational exposure to sulfur mustard and summarize the in vitro findings of genotoxicity and metabolic disruption in several cell lines. The authors summarize the compilation of human, animal and in vitro data, and their implications for long-term health consequences are presented.

Weese provides a comprehensive review of measured association between putative environmental exposures during the Persian Gulf War and symptoms, reporting a clinical outcome, emphasizing the strength, if any, of measured relationships between solvents, smoke, pesticides, pyridostigmine bromide, and chemical warfare agents and specific conditions. Furthermore, this chapter provides an in-depth discussion of problems associated with the definition of cohorts, the use of data from Gulf War Registries, the problem of case definition, and the uncertain nature of putative exposures.

Borowitz, Isom, and Baskin describe key pathologic sequelae to acute and chronic exposure to cyanide. They provide exposure and risk assessment, with emphasis on the effects of cyanide on the neural tissue. These effects are primarily characterized as effects of cyanide on the metabolism of neurons, cyanide and oxidative stress in neuronal cells, cyanide-induced hyperpolarization, and neuronal activation by cyanide, processes which implicate abnormal sodium channel function in cyanide-induced neuronal damage. Endogenous generation of cyanide in neuronal tissue is also postulated as a causal mechanism in disease. Problems in metabolism of cyanide leading to chronic, low-level exposure are described and discussed.

Salem, Olajos, and Katz provide a historical overview of the testing and development of riot-control agents by the military forces of several nations, including the United States. They distinguish between riot-control agents as military chemicals vs. chemical warfare agents (such as nerve agents, blister agents, choking agents, blood agents, and incapacitating agents). Riot-control agents include three subclasses—lacrrimators, sternutators, and vomiting agents—based on their salient physiological effects. Ocular, cutaneous, genotoxic, carcinogenic, and human toxicologic effects are provided for relevant instances of each of these classes of riot-control agents.

Adler, Oyler, Keller, and Lebeda provide an overview of botulinum neurotoxin action leading into a description of the syndrome known as botulism and a discussion of possible treatment options. Subsequently, Adler et al. develop purported terrorist or military anticipated use of botulinum neurotoxin and the threat thereof. This threat of use has led to investments in research that have achieved several major milestones.
and provided insights into mechanisms of action and a resolution of crystal structure. These authors suggest future promising areas of research into this problem. They end with brief discussions of some recent research success, viz., inhibitors of toxin binding, inhibitors of internalization, and inhibitors of translocation, providing examples in each case.

Romano and King suggest likely psychological, physiological, and neurobehavioral effects that may be encountered if chemical warfare agents are employed against U.S. forces, or even more troublesome, against U.S. citizens. They also describe the implications for health care if either these agents or their medical countermeasures are employed. Furthermore, because these pharmacologic and toxicologic actions could occur in the broad context of a nuclear, biological, or chemical environment with attendant confounding variables, they perhaps could lead to increased difficulty in the differential diagnosis of stress reaction vis-a-vis organophosphate-induced organic brain syndromes.

Moore and Alexander describe the organization and capabilities of the national response apparatus to a domestic or international terrorist use of a “weapon of mass destruction.” This apparatus involves many federal agencies that support and complement local and state response systems which respond to such incidents. The review also discusses the implications of “low-level toxicity of chemical warfare agents” for the crisis and consequence management phases of the federal response. Finally, the authors provide a brief summary of how several federally funded research and development programs may enhance future response capabilities.

The editors wish to thank Ms. Patricia Little whose persistence, attention to detail, and sense of purpose kept the editors and many of the contributors on track. We also wish to thank her Springfield, IL counterpart, Ms. Judith M. Bryan. Without the efforts of these two individuals, this work would not have proceeded on schedule.

The editors wish to thank also Colonel James Little, Commander of the U.S. Army Medical Research Institute of Chemical Defense, for his support of the overall initiative, Dr. James King, who steadfastly pushed us in pursuit of scholarly excellence, and the contributors for submitting their work in a timely fashion and for making the necessary modifications. The Medical Research Institute of Chemical Defense is the Army’s lead laboratory for the development of medical countermeasures to chemical warfare agents. It functions as a subordinate command of the U.S. Army Medical Research and Materiel Command, Ft. Detrick, MD. The editors thank Dr. Carl J. Getto, Dean and Provost, for his encouragement to publish this book. We also express gratitude to the reviewers who are identified in the acknowledgments in each chapter.

Finally, the editors wish to thank Candy Romano and Shipra Somani for their patience and encouragement. Without them, this task would have been onerous; with their support, it was an enriching experience.

© 2001 by CRC Press
Contributors

Dr. Michael Adler
Commander
U.S. Army Medical Research Institute
of Chemical Defense
ATTN: MCMR-UV-PN (Dr. Adler)
3100 Ricketts Point Rd.
Aberdeen Proving Ground, MD
21010-5400

Steve M. Alexander
Program Manager
Domestic Preparedness
Battelle Memorial Institute
2012 Tollgate Rd., Suite 206
Bel Air, MD 21015

Yacov Ashani, Ph.D.
Israel Institute of Biological Research
Ness-Ziona
Israel

Steven I. Baskin, Ph.D., Pharm.D.
Commander
U.S. Army Medical Research Institute
of Chemical Defense
ATTN: MCMR-UV-PB (Dr. Baskin)
3100 Ricketts Point Rd.
Aberdeen Proving Ground, MD
21010-5400

Hendrik P. Benschop, Ph.D.
Manager, Department of Chemical
Toxicology
TNO Prins Maurits Laboratory
2280AA Rijswijk
The Netherlands

Joseph L. Borowitz, Ph.D.
Professor of Pharmacology and
Toxicology
Department of Medicinal Chemistry
and Molecular Biology
1021 Hovde Hall
Purdue University
West Lafayette, IN 47907-1021

Clarence A. Broomfield, Ph.D.
Commander
U.S. Army Medical Research Institute
of Chemical Defense
ATTN: MCMR-UV-PB (Dr. Broomfield)
3100 Ricketts Point Rd.
Aberdeen Proving Ground, MD
21010-5400

Douglas M. Cerasoli, Ph.D.
Commander
U.S. Army Medical Research Institute
of Chemical Defense
ATTN: MCMR-UV-PB (Dr. Cerasoli)
3100 Ricketts Point Rd.
Aberdeen Proving Ground, MD
21010-5400

Leo P. A. De Jong, Ph.D.
Department of Chemical Toxicology
TNO Prins Maurits Laboratory
2280AA Rijswijk
The Netherlands

Bhupendra P. Doctor, Ph.D.
Director, Division of Biochemistry
Walter Reed Army Institute of Research
503 Robert Grant Road
Silver Spring, MD 20910-7500

© 2001 by CRC Press
David E. Lenz, Ph.D.
Commander
U.S. Army Medical Research Institute
of Chemical Defense
ATTN: MCMR-UV-PB (Dr. Lenz)
3100 Ricketts Point Rd.
Aberdeen Proving Ground, MD
21010-5400

Donald M. Maxwell
U.S. Army Medical Research Institute
of Chemical Defense
ATTN: MCMR-UV-PB (Mr. Maxwell)
3100 Ricketts Point Rd.
Aberdeen Proving Ground, MD
21010-5400

John H. McDonough, Ph.D.
U.S. Army Medical Research Institute
of Chemical Defense
ATTN: MCMR-UV-PA (Dr. McDonough)
3100 Ricketts Point Rd.
Aberdeen Proving Ground, MD
21010-5400

David H. Moore, D.V.M., Ph.D.
Director
Medical Toxicology Programs
Battelle Memorial Institute
2012 Tollgate Rd., Suite 206
Bel Air, MD 21015

George Oyler, Ph.D.
Department of Neurology
University of Maryland School of
Medicine
Baltimore, MD 21201

James A. Romano, Jr., Ph.D.
Commander
U.S. Army Medical Research Institute
of Chemical Defense
3100 Ricketts Point Rd.
Aberdeen Proving Ground, MD
21010-5400

Harry Salem, Ph.D.
Director
Edgewood Chemical and Biological
Center
ATTN: AMSSB-RRT (Dr. Salem)
5183 Blackhawk Rd.
Aberdeen Proving Ground, MD
21010-5424

Ashima Saxena, Ph.D.
Division of Biochemistry
Walter Reed Army Institute of Research
503 Robert Grant Road
Silver Spring, MD 20910-7500

Robert Sheridan, Ph.D.
Commander
U.S. Army Medical Research Institute
of Chemical Defense
ATTN: MCMR-UV-PN (Dr. Sheridan)
3100 Ricketts Point Rd.
Aberdeen Proving Ground, MD
21010-5400

Frederick R. Sidell, M.D.
14 Brooks Rd.
Bel Air, MD 21014

© 2001 by CRC Press
# Table of Contents

## Chapter 1
**Health Effects of Low-Level Exposure to Nerve Agents**
*James A. Romano, Jr., John H. McDonough, Robert Sheridan,* and *Frederick R. Sidell*

## Chapter 2
**Toxicokinetics of Nerve Agents**
*Hendrik P. Benschop and Leo P. A. DeJong*

## Chapter 3
**Low-Level Nerve Agent Toxicity under Normal and Stressful Conditions**
*Satu M. Somani and Kazim Husain*

## Chapter 4
**Blood-Brain Barrier Modulations and Low-Level Exposure to Xenobiotics**
*Hermona Soreq, Daniela Kaufer, Alon Friedman,* and *David Glick*

## Chapter 5
**Pharmacokinetics and Pharmacodynamics of Carbamates under Physical Stress**
*Satu M. Somani, Kazim Husain,* and *Ramesh Jaganathan*

## Chapter 6
**New Approaches to Medical Protection against Chemical Warfare Nerve Agents**
*Bhupendra P. Doctor, Donald M. Maxwell,* *Yacov Ashani,* *Ashima Saxena,* and *Richard K. Gordon*

## Chapter 7
**Nerve Agent Bioscavengers: Protection against High- and Low-Dose Organophosphorus Exposure**
*David E. Lenz,* *Clarence A. Broomfield, Donald M. Maxwell,* *Ashima Saxena,* and *Douglas M. Cerasoli*

## Chapter 8
**Chronic Effects of Acute, Low-Level Exposure to the Chemical Warfare Agent Sulfur Mustard**
*Charles G. Hurst* and *William J. Smith*

© 2001 by CRC Press
1 Health Effects of Low-Level Exposure to Nerve Agents*

James A. Romano, Jr., John H. McDonough, Robert Sheridan, and Frederick R. Sidell**

CONTENTS

I. Introduction
II. Chronic Health Effects of Acute Exposure
III. Chronic Health Effects of Repeated Low-Level Exposure
IV. The Contributions of *In Vitro* Studies to Determine Health Effects of Low-Level Exposure
V. Summary and Conclusions
References

I. INTRODUCTION

Nerve agents are highly toxic organophosphorous (OP) compounds that are chemically related to some insecticides (parathion, malathion). The four most common nerve agents are tabun (o-ethyl N,N-dimethyl phosphoramidocyanidate; military designation, GA), sarin (isopropyl methyl phosphonofluoridate; military designation, GB), soman (pinacolyl methyl phosphonofluoridate; military designation, GD), and VX (o-ethyl S-2-N,N-diisopropylaminoethyl methyl phosphonofluoridate). These compounds exist as colorless and relatively odorless liquids and are meant for use in weapon systems (shells, rockets, bombs) that are designed to deliver them as aerosols or fine sprays. They exert their toxic effects by inhibiting the cholinesterase (ChE) family of enzymes to include acetylcholinesterase (AChE; E.C.3.1.1.7), a critically important central nervous system (CNS) and peripheral nervous system (PNS) enzyme that hydrolyzes the neurotransmitter acetylcholine (ACh). Although the nerve agents can inhibit other esterases, their potency and specificity for inhibiting AChE account for their exceptionally high toxicity. For example, the rate constants for inhibition of AChE by soman, sarin, tabun, or VX are two to three orders of magnitude greater than for the more commonly known OP compounds such as DFP, paraoxon, or methylparaoxon.† Likewise, the rate constants for inhibition of AChE by

* The opinions or assertions contained herein are the private views of the authors, and are not to be construed as reflecting the view of the Department of the Army or the Department of Defense.
** All authors contributed equally.

© 2001 by CRC Press
the nerve agents are also two to five times greater than for trypsin (E.C.3.4.21.4), chymotrypsin (E.C.3.4.21.1), or carboxylesterase (E.C.3.1.1.1), indicative of selective inhibition of this enzyme.

Nerve agents bind to the active site of the AChE enzymes, thus preventing them from hydrolyzing ACh. The enzyme is inhibited irreversibly, and the return of esterase activity depends on the synthesis of new enzyme (~1–3% per day in humans). All agents are highly lipophilic and readily penetrate the CNS. Acetylcholine is the neurotransmitter at the neuromuscular junction of skeletal muscle, the preganglionic nerves of the autonomic nervous system, the postganglionic parasympathetic nerves, as well as muscarinic and nicotinic cholinergic synapses within the CNS. Following nerve agent exposure and the inhibition of ~60% of the AChE enzyme pool, levels of ACh rapidly increase at the various effector sites resulting in continuous overstimulation. It is this hyperstimulation of the cholinergic system at central and peripheral sites that leads to the toxic signs of poisoning with these compounds. The signs of poisoning include miosis (constriction of the pupils), increased tracheobronchial secretions, bronchial constriction, increased sweating, urinary and fecal incontinence, muscle fasciculations, tremor, and convulsions/seizures of CNS origin and loss of respiratory drive from the CNS. The relative prominence and severity of a given sign are highly dependent on the route and degree of exposure. Ocular and respiratory effects occur rapidly and are most prominent following vapor exposure, while localized sweating, muscle fasciculations, weakness, paralysis, and gastrointestinal disturbances are the predominant signs following percutaneous exposures and usually develop in a more protracted fashion. The acute lethal effects of the nerve agents are generally attributed to respiratory failure caused by a combination of effects at both central and peripheral levels and are further complicated by copious secretions, muscle fasciculations, and convulsions. There are several excellent reference sources that provide more detailed discussions of the history, chemistry, physiochemical properties, pharmacology and toxicology of nerve agents.

Human estimates of nerve agent toxicity have been derived from animal studies. They range from 7 μg/kg (VX) to 80 μg/kg (tabun) as the LD₅₀ for the i.v. route of administration, while the percutaneous LD₅₀ for tabun is estimated at 1000 mg, 1700 mg for sarin, 100 mg for soman, and 10 mg for VX, for a 70 kg person, respectively. The rapid onset of effects and extreme toxicity have made these compounds eminently suitable for use as chemical warfare (CW) agents, and in some cases, many thousands of tons of these agents have been synthesized for military use. Exposure to lethal levels of nerve agents will produce toxicities that are precipitate in onset and catastrophic in effect. For these reasons, major medical research efforts since the 1940s have focused on developing the best possible lifesaving therapeutic interventions, pretreatments, or, more recently, prevention of long-term changes in CNS function following a moderate to severe intoxication, using anticonvulsant drugs.

Due to the focus on lifesaving interventions, it was not until the early 1980s that the question of chronic health effects of low-level exposure to nerve agents was
subjected to its first major review. The Committee on Toxicology, National Academy of Sciences, studied the available literature reports from the soldier-volunteer test program of the former Army Chemical Center at the then Edgewood Arsenal, now a part of Aberdeen Proving Ground, MD. \textsuperscript{10} Soldier-volunteers participated in this test program from 1958 to 1975. There were 15 anticholinesterases (anti-ChE) tested on approximately 1400 subjects during this timeframe, with the great majority of anti-ChE agents being tested during the 1950s and 1960s.

The National Academy of Sciences review found that mortality data compiled in 1981 did not indicate increased deaths among soldier-volunteers when compared to comparable soldiers outside the testing program. There was no clear-cut indication of long-lasting CNS effects and no evidence for mutagenicity, carcinogenicity, male reproductive, or cataractogenic effects.\textsuperscript{10} The National Academy of Sciences review committee also reported confidence that its analyses would have had the power to detect any major health effects, had they been present. In general, that viewpoint was considered to be “state-of-the-art,” with very little contention until the appearance of Persian Gulf War Illness in the early 1990s.

As a result of concern regarding a high incidence of undiagnosed illness among veterans of Operation Desert Shield/Storm, a Presidential Advisory Committee was formed to analyze the full range of the Federal Government’s outreach, medical care, research, and coordinating activities pertinent to Gulf War Veterans’ Illness (GWI). The Presidential Advisory Committee also looked at short- and long-term health effects of selected Gulf War risk factors, e.g., chemical/biological weapons, depleted uranium, infectious diseases, vaccines against potential biological warfare agents, pyridostigmine bromide, etc. The Presidential Advisory Committee gave specific and serious attention to the question of health effects of low-level exposure to nerve agents. Their conclusions could be summarized as follows:

1. Available scientific evidence does not indicate that long-term, subtle, neuropsychological and neurophysiological effects could occur in humans following low-level (asymptomatic) exposure.
2. The amount of data from either human or animal research on low-level exposures is minimal.
3. To close this gap in the current knowledge base, the Department of Defense was urged to support additional research on the long-term health effects of low-level exposures to CW agents, the nerve agents in particular.\textsuperscript{11} Such an increased level of research has already been initiated, and some elements of it are discussed throughout this chapter.

Because of the great national interest, and perhaps because of technological advances in allowing public access to data, the current status of the federal portfolio of research in this area is readily available through the Internet. The current, annually updated summaries of progress in this research area of vital national interest can be found at \url{http://www.va.gov/resdev/}. This website is the Internet-based version of the Department of Veterans’ Affairs Annual Report to Congress on Federally Sponsored
Research on Gulf War Veterans' Illness for 1998. At this site, one can find an “Overview of the Federal Research Program” (see Appendix D). Among the long-term research recommendations of that overview are the following:

- Development of exposure biomarkers for CW agents
- Development of a strategic research plan for investigating the long-term health effects of exposure to low concentrations of CW agents. The author of the overview notes that these recommendations have been guiding the selection of new research projects since November 1996. The Annual Report to Congress for 1998 lists 10 research projects whose primary focus relates to CW agent exposure and health effects.\(^\text{12}\)

Another impetus for renewed investigations in this area is based on the question, “Is the current United States military medical treatment doctrine, as well as physical protective measures (protective masks, clothing, and support systems), adequate to protect soldiers in future deployments from effects of exposure to low levels of CW agents?”\(^\text{13}\)

Two recent reviews of the literature on potential long-term health effects from low-level exposure to nerve CW agents have presented slightly different analyses of this issue and, not surprisingly, they have reached slightly different conclusions. Brown and Brix\(^\text{14}\) argued that for nearly all accidental or wartime exposures to nerve agents or OP compounds, it is difficult to obtain reliable exposure data. Thus, they argued that exposures could be characterized as high, intermediate, or low, depending upon factors such as intensity of cholinergic signs (e.g., rhinorrhea, salivation, neuromuscular effects, etc.), level of ChE inhibition, and type of medical treatment required. Clearly identified long-term effects have been noted at or above their defined Intermediate Level Exposure. Long-term health effects, according to Brown and Brix,\(^\text{14}\) are not reported in individuals experiencing repeated low-level exposure alone.

In his brief review of chronic effects of low-level exposure to anticholinesterases, Roy\(^\text{15}\) concluded that “Concerns about major adverse health effects of low-level exposure to anticholinesterases in general seem entirely unwarranted on the basis of currently available literature, but the data are at present insufficient to reflect the possibilities of subtle, agent-specific effects.” In the section labeled “Chronic Health Effects of Repeated Low-Level Exposure,” we will also review the scientific basis for these health concerns.

It is common practice for toxicologists to differentiate exposure to chemicals based on the dose and the duration of exposure. Four timeframes have been used to define duration of exposures: acute, subacute, subchronic, and chronic. It is useful in light of today’s interest in “long-term, low-level” exposures to clarify these terms. Acute exposure is defined as exposure to a chemical for less than 24 h. Subacute exposure refers to an exposure of 1 month or less, subchronic for 1 to 3 months, and chronic for more than 3 months. These exposures can be by any route; for most chemicals it is the oral route with the chemical given in the diet.\(^\text{16}\) However, the limited animal studies using nerve agents have usually employed parenteral administration.
of the agent, and virtually all of them involve acute or subacute durations of exposure. All are intermittent, e.g., usually once a day. When referring to an inhalation exposure, the exposure duration most frequently used is 4h.

It is equally important to clearly define the term “low-level exposure.” This term has seen many different usages in the papers reviewed by these authors. These appear to range from any non-lethal exposure through “subtoxic” (defined by DeMenti as no clinical signs) to “subclinical” (defined by DeMenti as no clinical signs and no significant depression of ChE). Exposure, then, is any contact with a chemical that may induce a biochemical effect. Each definition suffers from arbitrariness and we see no way around this. For the purposes of this review, we will attempt to characterize each paper in terms of presence/absence of either clinical signs or symptoms (in the case of human studies), and level and type of ChE inhibition.

II. CHRONIC HEALTH EFFECTS OF ACUTE EXPOSURE

Much of the data regarding long-term neurological sequelae to exposures to cholinesterase inhibitors in man have been gathered following accidental exposures to organophosphate pesticides. While pertinent, extrapolation from these exposures to predictions of effects from nerve agents may be subject to risk. Several phenomena appear to differentiate nerve agent exposure from exposure to organophosphorus (OP) pesticides. These include:

1. The fact that the cholinergic crisis caused by acute, severe intoxication with the OP pesticides is generally much longer than that caused by OP nerve agents (days to weeks for pesticides vs. hours for nerve agents).
2. Many OP pesticides produce delayed peripheral neuropathy, a phenomenon known for more than 50 years, whereas nerve agents have caused polyneuropathy in animals only at doses manifold greater than the LD₅₀—a phenomenon only seen in the presence of massive pretreatment and therapy with atropine and oxime.
3. The “intermediate syndrome,” a delayed manifestation of OP poisoning seen in perhaps up to 100 accidentally poisoned patients, has not been described after administration of nerve agents to animals, nor in the instances of nerve agent poisoning in man.

Grob et al. described the effects of acute to subacute short-term exposure of humans to DFP (1–2 mg, IM, daily for up to 7 days) on electroencephalographic (EEG) and psychological parameters. The changes produced by DFP included increases in EEG potential, frequency (especially noted was an increase in beta rhythm), more irregularities in rhythm, and by the intermittent appearance of abnormal waves similar to those seen in patients with grand mal epilepsy (high voltage waves of 3 to 6 Hz, usually most marked in frontal leads, and increased by hyperventilation). The CNS symptoms noted were excessive dreaming, insomnia, jitteriness and restlessness, increased tension, emotional lability, subjective tremulousness,
nightmares, headache, increased libido, giddiness, drowsiness, paresthesias, mental confusion, and tremor. The EEG changes usually followed the onset of CNS symptoms. CNS symptoms and EEG changes were correlated with the depression of red blood cell ChE to 70 and 60% of original activity, respectively. Central nervous system symptoms disappeared within 1 to 4 days after exposure was stopped, while the EEG changes persisted in a diminishing degree from 8 to 42 days (average of 29 days). Essentially similar CNS symptoms and EEG changes were described by Holmes and Gaon as occurring acutely in OP-pesticide-exposed workers. They also noted that the more severely exposed individuals or those with multiple exposures, tended to display persistent symptoms that included forgetfulness, irritability, and confused thinking, although the duration of these persistent symptoms was never clearly defined.

These CNS symptoms and EEG changes are virtually identical to those that have been reported to occur following symptomatic exposure to different nerve agents. Grob and Harvey described extensive studies of the effects of sarin in man, to include effects on ChE, EEG, and behavior. They noted behavioral and EEG effects virtually identical to those reported for DFP. These effects began coincident with the depression of plasma and red blood cell ChE activity to approximately 60 and 50% of original activity, respectively, following a single i.v. dose, or 34 and 22% of original activity, respectively, following oral administration. These differences between i.v. and oral administration of sarin suggest that the rate of ChE inhibition, and consequently the rate of increase in CNS ACh, are important factors in the development of symptoms of exposure. Bowers et al. studied the effects of the nerve agent VX in man and described behavioral symptoms of anxiety, psychomotor depression, a general intellectual impairment consisting of difficulties in concentration and retention, and sleep impairments generally involving insomnia due to excessive dreaming.

Psychological/behavioral effects were typically evident before the occurrence of physical symptoms. These effects were associated with whole blood ChE inhibitions of > 60%. There have been descriptions of the acute toxic effects in humans that follow high-dose exposure (≥ LD₅₀) to the nerve agents soman, sarin, and VX. The same cluster of behavioral symptoms that are reported following lower doses (anxiety, psychomotor depression, intellectual impairment, sleep disturbances) dominate the clinical picture in the immediate period following resolution of the acute toxic signs of intoxication and then slowly fade with time, sometimes taking months to fully resolve.

There have been a number of investigations as to the possible long-term consequences of an acute symptomatic exposure to OP compounds. For the nerve agents, Burchfiel et al. evaluated the long-term effects of an acute high dose (5 µg/kg, i.v.) of sarin on the EEG of rhesus monkeys. The animals were paralyzed and artificially respirated during exposure since this dose of sarin produced generalized seizure activity on the EEG that lasted an average of 2.5 h. At both 24 h and 1 year following the exposure, there was a significant increase in the relative voltage in the beta frequency bands (13–22 Hz = beta-1; 22–50 Hz = beta-2) in the occipital-temporal EEG lead while the animals were awake in darkness. Similar EEG effects were seen in other animals in this study that were exposed to high doses of the chlorinated
hydrocarbon, dieldrin. Functional behavioral tests of other rhesus monkeys exposed to sarin under identical conditions revealed no deficits in performance of a previously learned delayed response test 24 h after the exposure. Duffy et al. performed a similar analysis of EEG of munitions workers accidentally exposed to the nerve agent sarin at doses that produced clinical signs and symptoms of exposure and produced a reduction of erythrocyte ChE at least 25% below the individual’s pre-exposure baseline. Within the exposed group, there was a maximally exposed subgroup that had experienced three or more such exposures. The study was performed at least one year after the last exposure. Univariate and multivariate analysis of the data show that the exposed group, especially the maximally exposed subgroup, displayed:

1. Elevated amounts of spectral energy in high-frequency beta activity
2. Visual inspection of the EEG showed decreased amounts of alpha (9–12 Hz) activity along with increased amounts of slow activity (0–8 Hz, delta and theta) and an increased amount of “nonspecific” abnormalities in the EEG background.
3. Increased amounts of rapid eye movement (REM) sleep.

The functional consequences of these EEG changes were not established, but this group reportedly had a high incidence of self-reported memory disturbances, difficulty maintaining alertness and appropriate focusing of attention.

Several studies of the long-term effects of the sarin-exposure victims from Japan have been published. Yokoyama et al. evaluated 18 victims of the Tokyo subway incident 6 to 8 months after exposure. All but three of these victims had plasma ChE values below normal values on the day of exposure. Sarin-exposed individuals scored significantly lower than controls on a digit symbol substitution test; they scored significantly higher than controls on a general health questionnaire (GHQ; psychiatric symptoms) and a profile of mood states (POMS; fatigue). Additionally, they had elevated scores on a post-traumatic stress disorder (PTSD) checklist; they had significantly longer P300 latencies on event-related brain-evoked potentials and longer P100 latencies on brain visual-evoked potentials; and female exposed cases had significantly greater indexes of postural sway. The elevated scores on the GHQ and POMS were positively related to the increased PTSD scores and were considered to be due to PTSD. Nakajima et al. performed a cohort study of victims of the Matsumoto City sarin exposure 1 and 3 years following the incident. At 1 year following the exposure, they report that 20 victims still felt some symptoms (fatigue, asthenopia, blurred vision, asthenia, shoulder stiffness, and husky voice), and they had lower erythrocyte ChE activity than those who did not have symptoms and had all lived close to the sarin release site. (Note: Not all the symptoms seen at 1 year have been related to nerve agent exposure historically.) At 3 years, some victims still complained of experiencing these symptoms, although with a reduced degree and frequency. There have been two brief reports of severely poisoned nerve agent victims (one sarin, one VX) in Japan who experienced retrograde amnesia, possibly due to prolonged periods of seizures and/or hypoxia. Additionally, one of the Matsumoto victims who experienced prolonged seizure activity was followed for at least 1 year.