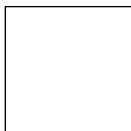


CHEMICAL WARFARE AGENTS: TOXICITY AT LOW LEVELS

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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.

Somani, 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—lacrimators, 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-à-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.

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