2012 Review on the Extension of the AMedP-8(C) Methodology to New Agents, Materials, and Conditions

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About This Publication
This work was conducted under contract DASW01-04-C-0003, Task CA-6-3079, “CBRN Casualty Estimation Update of the Medical CBRN Defense Planning & Response Project,” for the Joint Staff, Joint Requirements Office for CBRN Defense and the U.S. Army Office of the Surgeon General. The views, opinions, and findings should not be construed as representing the official position of either the Department of Defense or the sponsoring organization.

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Executive Summary

In 2005, the Institute for Defense Analyses (IDA) began developing a methodology for the North Atlantic Treaty Organization (NATO) to estimate casualties from chemical, biological, radiological, and nuclear (CBRN) weapons. The final draft documenting this methodology was published by IDA in 2009 and was promulgated by NATO in March 2011 as Allied Medical Publication 8 (C): NATO Planning Guide for the Estimation of CBRN Casualties, (AMedP-8(C)). Because AMedP-8(C) included a limited number of CBRN agents and effects, IDA has been asked each year since 2009 by the Army Office of the Surgeon General (OTSG) to review published literature to evaluate how the AMedP-8(C) methodology can be updated and expanded to new agents, materials, and conditions. This document, the 2012 review, fulfills both OTSG and NATO requirements and is the fourth in a series of annual reviews, updated as the scope of AMedP-8(C) expands. This annual review focuses primarily on newly available data that can be used to update existing agents or effects in the methodology.

This review is structured into four chapters. The introductory chapter states the objective of the 2012 annual review as well as the task requirements it fulfills. It also briefly introduces the AMedP-8(C) casualty estimation methodology and summarizes the past annual reviews and subsequent programs of work completed by IDA. Chapter 2, “The 2012 Review,” describes the literature review process and reports the major findings by agent. When new data could be used to update or extend the AMedP-8(C) methodology, the level of effort to incorporate these data or perform follow-on analyses was estimated in Chapter 3. Finally, Chapter 4 recommends topics for future analysis identified in this and prior reviews.

The key findings of the literature review, summarized by agent in Chapter 2, focus on human cases of exposure, advancements in medical countermeasure development, and response data from animal models. These summaries serve two purposes: 1) to identify data sources immediately useful to updating AMedP-8(C) human response parameters or otherwise modifying the methodology and 2) to help inform future analyses and to serve as a starting point for related research efforts.

The literature review revealed three different categories of work that could be carried out to update or extend the AMedP-8(C) methodology: editorial changes to the text of future versions of AMedP-8 or related documents, the incorporation of new data into existing AMedP-8(C) models, and the comparison of AMedP-8(C) models to other published models for validation or revision. Estimates for the level of effort required to complete future analyses identified in this review were based on IDA’s prior experiences performing analyses in this field.
Based on IDA’s understanding of the available literature and the needs of the sponsor, the IDA research team recommends a number of future efforts related to *AMedP-8(C)* human response modeling.

1. As a NATO document, *AMedP-8(C)* is subject to a periodic review every three years. Since its 2011 publication, the *AMedP-8(C)* methodology has been expanded to include human response parameters for additional agents and the consideration of medical care. Given these significant advancements, IDA recommends that a new version of *AMedP-8* be proposed at the 2014 review. The proposal should include incorporating, at a minimum, the new agents, the impact of medical care, and any editorial changes to keep the content current as described in this document.

2. During this review, the IDA team was successful in identifying new sources of data relevant to updating the *AMedP-8(C)* methodology. In particular, data are available that could impact the anthrax, botulism, brucellosis, glanders, plague, Q fever, smallpox, and tularemia models. In addition, IDA continues to pursue access to the human response studies conducted through the military research volunteer (MRV) program in the 1950s and 1960s, which could provide data useful to the Q fever, SEB, and tularemia models. IDA should conduct cost-benefit analyses to determine whether the new data would significantly improve the military medical planning process and warrant changes to the *AMedP-8(C)* methodology.

3. The IDA team should quantify the impact on the casualty estimate of radioprotectant drugs, radiation mitigators, and radiation therapeutic agents in NATO member national inventories or those in procurement, but not fielded. As many of these countermeasures are Food and Drug Administration-approved or have emergency use investigational new drug (IND) status, some efficacy data must be available.

4. Case histories from the SEARCH (System for Evaluation and Archiving of Radiation accidents based on Case Histories) radiation effects database should be reviewed to assess their value in validating or revising the *AMedP-8(C)* radiological agent human response models. In addition to requesting access to the SEARCH database, IDA should reach out to and collaborate with the Group to Link nonhuman Primate and Human radiation effects (GLiPH), which is leveraging the SEARCH data to establish correlations between human and non-human primate radiation exposures. With a better understanding of the GLiPH team’s efforts, IDA can determine how their work might fit within the framework of the *AMedP-8(C)* methodology.

5. The IDA team should compare the *AMedP-8(C)* dose-response models to the alternative dose-response models discovered in this literature review and any other published models. In particular, alternative dose-response models specific to anthrax and radiation were discovered, as well as a more general method of pooling infectivity data from multiple species. Analyses should be conducted to compare each alternative
methodology with the existing models within *AMedP-8(C)*. The result of these analyses should be a recommendation to continue with the current methodology or to change it, along with an estimate of the level of effort required to do so.

6. Many chemical and biological agents of interest to various government agencies are candidates for future inclusion in *AMedP-8(C)*. Levels of effort to incorporate more than 40 agents into the *AMedP-8(C)* methodology were estimated in the 2009 review, yet only a small fraction has since been modeled. IDA should develop a prioritization scheme for future inclusion of the remaining agents in *AMedP-8(C)* based on an analysis of the military threat or capability to NATO nations and the availability of modeling data for each agent.

7. As discussed in prior annual reviews, IDA stands ready to investigate the feasibility of incorporating the estimation of psychological casualties into the *AMedP-8(C)* methodology if and when this becomes a sponsor priority.
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1. Introduction

A. Objective

In 2005, the Institute for Defense Analyses (IDA) began developing a methodology for the North Atlantic Treaty Organization (NATO) to estimate casualties from chemical, biological, radiological, and nuclear (CBRN) weapons. The final draft documenting this methodology was published by IDA in 2009 and was promulgated by NATO in March 2011 as Allied Medical Publication 8 (C): NATO Planning Guide for the Estimation of CBRN Casualties, (AMedP-8(C)). Because AMedP-8(C) included a limited number of CBRN agents and effects, IDA has been asked each year since 2009 by the Army Office of the Surgeon General (OTSG) to review published literature to evaluate how the AMedP-8(C) methodology can be updated and expanded to new agents, materials, and conditions. This 2012 annual review focuses primarily on newly available data that can be used to update existing agents or effects in the methodology. IDA’s literature review included new and updated data sources for currently modeled CBRN agents and effects, novel medical countermeasures, and alternative human response models.

This review is in four chapters. This introductory chapter states the objective of the 2012 annual review as well as the requirements it fulfills. It also briefly introduces the AMedP-8(C) casualty estimation methodology and summarizes past annual reviews and subsequent programs of work completed by IDA. Chapter 2, “The 2012 Review,” describes the literature review process and reports the major findings by agent. When new data could be used to update or extend the AMedP-8(C) methodology, the level of effort to incorporate these data or perform follow-on analyses was estimated in Chapter 3. Finally, Chapter 4 recommends topics for future analysis identified in this and prior reviews.

B. Task Requirements

This document describes analysis completed under Task Order CA-6-3079 “CBRN Casualty Estimation Update of the Medical CBRN Defense Planning and Response Project,” Subtask 2 “Update Agents/Materials into AMedP-8(C) Methodology.” The task order specifies a “draft program of work identifying agents, effects, materials, and conditions of interest to the DOD [Department of Defense] (and NATO and other Federal agencies, as requested), but not currently included in AMedP-8(C).” This document is not an addendum to AMedP-8(C), but may be considered a supplement to the AMedP-8(C) Technical Reference Manual.¹

C. Background

*AMedP-8(C)* describes a general methodology that military planners use to estimate casualties from CBRN weapons. The annexes to *AMedP-8(C)* define specific modeling parameters for three chemical agents (sarin (GB), methylphosphonothioic acid (VX), and distilled mustard (HD)), five biological agents (those that cause anthrax, botulism, pneumonic plague, smallpox, and Venezuelan equine encephalitis (VEE)), seven radioisotopes (\(^{60}\)Co, \(^{90}\)Sr, \(^{131}\)I, \(^{137}\)Cs, \(^{192}\)Ir, \(^{238}\)Pu, and \(^{241}\)Am), and acute nuclear blast, radiation, and thermal effects.

The *AMedP-8(C)* methodology depends on a national transport and dispersal model to specify the amount of CBRN agent or effect where individuals in the scenario are located. The methodology then characterizes human response to exposure as a stepwise function of injury severity over time (called an injury profile). Based on the available toxicity data for chemical, radiological, and nuclear agents and effects, clinically distinguishable dose/dosage/insult ranges are developed for each agent or effect, and injury profiles are drawn for all ranges. Individuals are considered casualties at the time the injury profile first reaches a user-defined injury severity level.

For biological agents, the following five submodels are combined to determine the number of casualties over time.

1. The infectivity submodel estimates the number of individuals that become ill as a function of inhaled dose of agent.
2. The incubation period submodel estimates the time from exposure to the onset of symptoms.
3. The duration of illness submodel estimates the time from onset of symptoms to either death or recovery.
4. The disease profile submodel divides the illness into clinically differentiable stages and assigns each an injury severity level.
5. The lethality submodel estimates the number of individuals that die.

Just like for the chemical, radiological, and nuclear methodologies, individuals are considered casualties when the symptoms from a biological agent exposure (as defined by the disease profile submodel) reach a user-defined threshold.

With the exceptions of prophylaxis for anthrax, plague, and smallpox, *AMedP-8(C)* does not consider the effects of medical countermeasures on the casualty estimate. This was due to a restriction imposed by NATO that medical intervention be excluded from the methodology because it was not standardized across all NATO nations.
D. Past Reviews and Subsequent Program of Work

In 2009, the same year IDA published the final draft of AMedP-8(C), it was asked to nominate new agents to be considered for future versions of AMedP-8. The resulting analysis identified nearly 900 chemical and biological materials of concern to various governmental agencies. A representative subset of agents was further reviewed for availability of human response modeling data. Based on literature reviews, IDA estimated the level of effort required to extend the AMedP-8(C) methodology to include these new agents. This analysis, along with estimates of the level of effort to include psychological or civilian casualties, made up the 2009 Report on the Extension of the AMedP-8(C) Methodology to New Agents, Materials, and Conditions. This became the first in a series of annual reviews to update and expand the AMedP-8(C) methodology.

In the following year, IDA published the ratification draft of AMedP-8(C), as well as its technical reference manual, which documented the derivation of the underlying parameters. In addition, human response parameters were developed for five additional biological agents: staphylococcal enterotoxin B (SEB), and the causative agents of brucellosis, glanders, Q fever, and tularemia. The second annual review recommended extending the AMedP-8(C) methodology to include the impact of medical care and adding new agents to AMedP-8(C) to better align it with the Common User Database (CUD), a U.S. tool that estimates the medical requirements for different types of patients. Since the outputs of the AMedP-8(C) methodology are roughly equivalent to the inputs to the CUD, including the same CBRN agents and effects in both methodologies would benefit planners.

The 2011 program of work included modeling medical intervention for all CBRN agents and effects in AMedP-8(C) as well as for the five additional biological agents modeled in 2010.

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7 Carl A. Curling et al., The Impact of Medical Care on Casualty Estimates from Battlefield Exposure to Chemical, Biological and Radiological Agents and Nuclear Weapon Effects, IDA Document D-4465 (Alexandria, VA: IDA, March 2012); Carl A. Curling et al., Addenda to Allied Medical Publication 8, “NATO
The third annual review\textsuperscript{8} prioritized an analysis of the effect of bioscavengers on chemical nerve agents, the inclusion of historical data from experiments with military research volunteers (MRV) from the U.S. offensive weapons program, and the expansion of the methodology to include a number of additional agents of interest to the sponsor.

In 2012, IDA began developing human response modeling parameters for five new chemical agents (chlorine, cyanogen chloride, hydrogen cyanide, hydrogen sulfide, and phosgene) and seven new biological agents (ricin, T-2 mycotoxin, and the causative agents of eastern equine encephalitis, Ebola, Marburg, melioidosis, and western equine encephalitis). In addition, IDA investigated the potential use of bioscavengers to treat chemical injuries and sought access to the set of MRV exposure data for Q fever, SEB, and tularemia.

2. The 2012 Review

A. Approach

The 2012 review focused on identifying potential new data sources for the CBRN agents and effects in *AMedP-8(C)* and the subsequently published addenda on the five new biological agents and medical care. Explicitly excluded from the review was information related to the five chemical agents and seven biological agents still under development. IDA reviewed publicly available, peer-reviewed literature from 2009 to the present to identify relevant references published since the original development of the *AMedP-8(C)* human response parameters.

The primary collection of articles was gathered using EBSCOHost. For the initial literature search, the agent name for chemical agents and disease name for biological agents were used. Because the goal was to collect and review summary references that might serve as a starting point for future analyses, rather than identifying every potential change to *AMedP-8(C)*, the search results were filtered to reduce the number of articles to review. For example, there were more than 16,000 results for the term *anthrax* searched in “all text.” When narrowing the search terms to *anthrax* in “subject terms” and *review* in “abstract” and also filtering by language (English) and reference type (academic journals), the results were reduced to 76. In addition to the EBSCOHost searches, PubMed searches were performed specifically for recently published reports of animal (ideally non-human primate) models to characterize the pathology of disease or develop a median infective or lethal dose estimate.

To supplement the peer-reviewed journal articles with more recent outbreak information from around the world, reports were gathered from the Program for Monitoring Emerging Diseases (ProMED) website. In addition, articles and mortality tables of notifiable diseases from the Centers for Disease Control and Prevention’s (CDC) *Morbidity and Mortality Weekly Report (MMWR)* were useful in identifying recent cases in the United States. The U.S. Food and Drug Administration (FDA) website and ClinicalTrials.gov were also valuable resources for determining the status of novel medical countermeasures in development.

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9 See the EBSCOHost website at [http://www.ebscohost.com/](http://www.ebscohost.com/).


11 ProMED is “an Internet-based reporting system dedicated to rapid global dissemination of information on outbreaks of infectious diseases and acute exposures to toxins that affect human health, including those in animals and in plants grown for food or animal feed.” For more information, visit the ProMED website at [http://www.promedmail.org/](http://www.promedmail.org/).

12 See the U.S. Food and Drug Administration (FDA) website at [http://www.fda.gov/](http://www.fda.gov/).
The major findings of the literature review are summarized by agent in the following sections. Subjects of focus include human cases of exposure, advancements in medical countermeasure development, and response data from animal models. These summaries serve two purposes: 1) identifying data sources immediately useful to updating \textit{AMedP-8(C)} human response parameters or otherwise modifying the methodology and 2) helping to inform future analyses and serve as a starting point for related research efforts.

Topics related to the first purpose are highlighted in Chapters 3 and 4, where future analyses are recommended and the level of effort is estimated for each. On the other hand, data not currently applicable to the \textit{AMedP-8(C)} methodology, such as medical countermeasure test data for items not in national military inventories, are still worth capturing both to help anticipate which countermeasures may be fielded in the future, and to facilitate and expedite data collection and analysis if and when countermeasures are fielded. As another example, endemic disease data may be secondary to data from more controlled studies for deriving human response parameters, but descriptions of human disease can still benefit modeling, and variations in human disease rates may indicate changes in the agent or host that may become militarily relevant.

\section*{B. Biological Agents}

\subsection*{1. Anthrax}

\subsubsection*{a. Human Cases}

Since the appearance of anthrax-contaminated letters in October 2001, much of the attention on anthrax in the United States has focused on its use as a weapon of bioterrorism and the threat of an aerosolized attack. The current literature review highlighted the fact that anthrax is still endemic to many parts of the world, and natural human illness is almost always associated with direct or indirect contact with infected animals. More than 40 countries reported suspected or confirmed human anthrax cases since 2009, with no evidence of malicious intent in any case.\footnote{See the search results for posts with the keyword \textit{anthrax} since 2009 at http://www.promedmail.org/.} Of note were large-scale outbreaks of human anthrax that affected Bangladesh in recent years, with hundreds of people contracting cutaneous or gastrointestinal anthrax after butchering and consuming the meat of contaminated animals.\footnote{Apurba Chakraborty et al., “Anthrax Outbreaks in Bangladesh, 2009–2010,” \textit{American Journal of Tropical Medicine and Hygiene} 86, no. 4 (2012): 703–710; Muhammad Afser Siddiqui et al., “Recent Outbreak of Cutaneous Anthrax in Bangladesh: Clinico-Demographic Profile and Treatment Outcome of Cases Attended at Rajshahi Medical College Hospital,” \textit{BMC Research Notes} 5 (2012): 464.}
1) Injection Anthrax

In addition to the Bangladesh cases and a number of other cutaneous outbreaks in recent years, the literature review revealed several more unusual incidents with anthrax. For instance, recent anthrax outbreaks in Europe among heroin users infected through the injection of anthrax-contaminated drugs led to the definition of a new type of anthrax dubbed injection anthrax.\(^{16}\) This form of anthrax is associated with severe pain and swelling at the injection site, but the black eschars associated with cutaneous anthrax were notably absent.\(^{17}\) In an outbreak in 2009–2010, there were 47 laboratory-confirmed and an additional 72 suspected cases of injection anthrax in Scotland. Of the 119 Scottish cases, there were 14 reported deaths.\(^{18}\) There were also five confirmed anthrax cases in England and three in Germany.\(^{19}\) A second outbreak began in June 2012 and infected at least 15 heroin-injecting drug-users in Germany (1 death, 3 survivors), Denmark (1 death, 1 survivor), France (1 survivor), Scotland (1 death, 1 survivor), Wales (1 survivor), and England (4 deaths, 1 survivor) through March 2013.\(^{20}\) A comprehensive summary of the first outbreak in Scotland, including data on the clinical presentation of cases, was prepared by the Health Protection Scotland.\(^{21}\)


\(^{17}\) Hicks et al., “Overview of Anthrax Infection.”


\(^{19}\) Grunow et al., “Anthrax among Heroin Users.”


\(^{21}\) National Anthrax Outbreak Control Team, *Anthrax among Drug Users in Scotland*. 
2) U.S. Human Cases

Only two anthrax cases have been reported in the United States since 2009.\textsuperscript{22} The first was the 2009 gastrointestinal anthrax infection of a 24-year-old woman from a presumed aerosol exposure at an event using animal-hide drums in New Hampshire.\textsuperscript{23} The exact route of entry in this case is unclear, although it is suspected that the “spores were either relatively large or clumped and were aerosolized and then swallowed.”\textsuperscript{24} Alternatively, it is possible that the woman consumed food or water that was contaminated by aerosolized spores.\textsuperscript{25} Once the diagnosis of gastrointestinal anthrax was made, the patient was treated with intravenous (IV) anthrax immune globulin in addition to antibiotics and was only the fifth person in the world to receive this treatment.\textsuperscript{26} After nearly two months in the hospital, the patient was transferred to a rehabilitation facility and was discharged 20 days later.\textsuperscript{27}

The second American case was the 2011 inhalation anthrax infection in a Florida man on vacation in Montana, Wyoming, and the Dakotas.\textsuperscript{28} On 4 August, near the end of his trip, he became ill and was admitted to a hospital in Minnesota, where he was diagnosed with inhalational anthrax before being transferred to another hospital on 7 August. Like the New Hampshire woman, this patient was treated with anthrax immune globulin from the CDC (reportedly the 19th person to receive this treatment), which may have helped his recovery. Fluid was also drained from the patient’s lungs, which was reported to be essential to survival in a prior case of inhalational anthrax.\textsuperscript{29} On 29 August the patient was released from the hospital after more than three weeks of treatment. The source of exposure remains unknown, although it is suspected that he was exposed to spores in the soil during his vacation.

\begin{itemize}
  \item \textsuperscript{24} Klempner et al., “Case 25-2010.”
  \item \textsuperscript{25} Mayo et al., “Gastrointestinal Anthrax.”
  \item \textsuperscript{26} Klempner et al., “Case 25-2010.”
  \item \textsuperscript{27} Mayo et al., “Gastrointestinal Anthrax”; Klempner et al., “Case 25-2010.”
  \item \textsuperscript{28} Robert Roos, “Early Diagnosis, Treatment Helped Florida Man Beat Anthrax,” \textit{Center for Infectious Disease Research and Policy (CIDRAP) News} (30 August 2011), \url{http://www.cidrap.umn.edu/cidrap/content/bt/anthrax/news/aug3011anthrax.html}.
  \item \textsuperscript{29} James J. Walsh et al., “A Case of Naturally Acquired Inhalation Anthrax: Clinical Care and Analyses of Anti-Protective Antigen Immunoglobulin G and Lethal Factor,” \textit{Clinical Infectious Diseases} 44, no. 7 (2007): 968–971.
\end{itemize}
b. Medical Countermeasures

1) Anthrax Vaccines

In May 2012 the FDA approved an abbreviated primary dosing schedule for the currently licensed anthrax vaccine, BioThrax (formerly known as Anthrax Vaccine Adsorbed). The primary dosing schedule was changed from a five-shot series (at 0, 1, 6, 12, and 18 months) plus annual boosters to a three-shot series (at 0, 1, and 6 months) plus boosters at 12 and 18 months followed by annual boosters thereafter. This change reflects the evidence that protective antibody levels are achieved after the first three doses of BioThrax, although frequent boosters are still required to maintain protective levels.

Additional studies with BioThrax are also underway to test other uses for the vaccine. Participants are currently being recruited for a five-year clinical trial to determine any adverse effects of BioThrax administered to pregnant women and a Phase II study to determine the effect of a three dose series of BioThrax on the effectiveness of ciprofloxacin. A Phase III trial to demonstrate the effectiveness of a three-dose series of BioThrax as post-exposure prophylaxis has also been completed. In the meantime, although BioThrax is not FDA-approved for post-exposure prophylaxis, it has been used in a three-dose regimen along with the regular 60-day course of antibiotics under an investigational new drug (IND) protocol.

Anthrax vaccine research in children may also be forthcoming. In March 2013, the Presidential Commission for the Study of Bioethical Issues released its recommendations for pre- and post-event medical countermeasure research on anthrax vaccination in children. It outlined the circumstances in which it might be permissible to perform anthrax vaccination research in children and specified a preferred age de-escalation procedure that might infer that research on the next oldest age group poses minimal risk.

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Despite the proven efficacy of BioThrax, anthrax vaccine research is an ongoing and high priority effort, and a number of recent reports have summarized the latest developments.\(^{37}\) Briefly, anthrax lethality is attributed to two virulence factors: (1) the toxin comprised of three proteins (protective antigen (PA), lethal factor (LF), and edema factor (EF)) and (2) the capsule.\(^{38}\) Although live spore-based vaccines have been effective in preventing disease in animals and are still used in humans by some nations, such as those from the former Soviet Union, other nations favor acellular rather than whole-spore vaccines for fear of residual virulence.\(^{39}\) Both the UK Anthrax Vaccine Precipitated and the U.S. BioThrax are sterile, acellular vaccines with PA as the main protective component.\(^{40}\)

As a result of ongoing efforts to develop a second generation recombinant protective antigen (rPA)-based anthrax vaccine, a number of candidate rPA vaccines have already completed Phase I clinical trials to test for safety in humans. Among the rPA anthrax vaccine candidates advancing to or already undergoing Phase II (effectiveness) clinical trials are SparVax, an \textit{E. coli}-based rPA vaccine created by PharmAthene;\(^ {41}\) GC1109, a vaccine developed by the Green Cross Corporation;\(^ {42}\) and PreviThrax, a product of Emergent BioSolutions (the producer of the licensed BioThrax vaccine).\(^ {43}\) Emergent BioSolutions is also recruiting participants for a Phase II trial of another “next-generation” vaccine candidate, NuThrax, also

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\(^{39}\) Beierlein and Anderson, “New Developments in Vaccines.”


known as AV7909, which is made of BioThrax combined with a novel immunostimulatory compound, CPG 7909.\(^4^4\)

A variety of other vaccine approaches are also being explored including plant-based\(^4^5\) and synthetic peptide vaccines\(^4^6\) directed at specific domains of the PA protein and a technique encapsulating rPA with a particulate carrier.\(^4^7\) Other vaccine candidates are being developed that aim to improve the protection of a PA-based vaccine by targeting other components of the bacteria as well. Multi-component subunit vaccines have been developed that target PA in combination with spore antigens,\(^4^8\) LF,\(^4^9\) and poly-gamma-D-glutamic acid (PGA) capsule components.\(^5^0\) There are also efforts to develop vaccines comprised of killed but metabolically active (KBMA) whole bacterial cells.\(^5^1\)

The development of vaccines that protect against multiple pathogens represents another potential way forward. Based on pre-clinical studies, a dual vaccine candidate that inoculates against smallpox and anthrax “not only is superior in immunogenicity and efficacy in comparison with the currently licensed vaccines against smallpox and anthrax, but also remedies the inadequacies associated with such licensed vaccines.”\(^5^2\)

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\(^{4^6}\) Oscherwitz, Yu, and Cease, “Synthetic Peptide Vaccine.”


\(^{5^0}\) Deog-Yong Lee et al., “Poly-Gamma-D-Glutamic Acid and Protective Antigen Conjugate Vaccines Induce Functional Antibodies against the Protective Antigen and Capsule of *Bacillus anthracis* in Guinea-Pigs and Rabbits,” *FEMS Immunology and Medical Microbiology* 57, no. 2 (2009): 165–172.


2) Anti-Toxin Therapies

Post-exposure therapy with monoclonal antibodies has been another area of recent research with scientists developing antibodies against all three proteins (PA, LF, and EF) and the capsule, a number of which are being studied in human clinical trials. Raxibacumab (ABthrax), a human monoclonal antibody against rPA, has acquired FDA approval for use as a supplement to antibiotic therapy and is in the U.S. Strategic National Stockpile (SNS).

Another antibody therapeutic that is stored in the SNS is anthrax immune globulin (AIG), which is a polyclonal antibody derived from the pooled plasma of individuals vaccinated with BioThrax. AIG is considered an IND by the CDC, but has been used in combination with approved antibiotics to treat patients in the European injection anthrax cases, the 2011 Minnesota inhalation anthrax case, and the 2009 gastrointestinal case in New Hampshire.

c. Animal Models

For much of the last century, rhesus macaques were the primary non-human primate used in experiments with anthrax. In addition to the studies already identified in the technical reference manual for AMedP-8(C), a 2001 study to determine the inhalation anthrax LD50 in rhesus


56 Schneemann and Manchester, “Anti-Toxin Antibodies.”

57 Ramsay et al., “Outbreak of Infection with Bacillus anthracis.”

58 Roos, “Early Diagnosis, Treatment Helped Florida Man Beat Anthrax.”

59 Klempner et al., “Case 25-2010.”

macaques was recently ascertained. It identified four strains of \textit{B. anthracis} and calculated LD\textsubscript{50} values for each, which ranged from 6,700 to 40,100,000 spores. A 1995 document by Fritz et al., which characterizes the pathology of inhalation anthrax in rhesus macaques, may also contain information on the infectivity and lethality.

Recently, the FDA Animal Rule has spurred investigations into other non-human primate models of inhalation anthrax for FDA approval of therapeutics in humans. Publications based on this research revealed sources of additional data for use in calculating a median infective/lethal dose for the \textit{AMedP-8(C)} inhalation anthrax model.

The cynomolgus macaque model was most recently characterized in a 2012 study by Henning et al., which built off the prior work of Vasconcelos et al. The Vasconcelos study exposed 14 monkeys to aerosolized \textit{B. anthracis} and determined an LD\textsubscript{50} of 61,800 spores and a probit slope of 4.21. However, it is unclear how these values were calculated since all 14 monkeys died. Moreover, the dose data for the individual monkeys were not published other than specifying a range of 45,600 to 2,940,000 spores. The Henning group reported the inhaled doses for each of the 12 cynomolgus macaques exposed in their experiment (in terms of the LD\textsubscript{50} calculated by Vasconcelos et al.). All monkeys were exposed to hundreds of times the calculated LD\textsubscript{50}, yet two of them survived the challenge.

Other recent studies describe the pathology of inhalation anthrax in the African green monkey. One reports that the LD\textsubscript{50} for this species was previously determined to be 11,000 spores, although the data are unpublished. This same study challenged nine monkeys to doses ranging from 210,000 to 18,900,000 spores, and all succumbed except one monkey at the lowest dose. In a similar experiment, 12 monkeys with inhaled doses ranging from 200 to 10,000,000

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61 Roy Barnewall, James Estep, and Robert M. DeBell, \textit{Inhalation Median Lethal Dose (LD\textsubscript{50}) Determinations in Rhesus Monkeys Exposed to Bacillus anthracis} (Columbus, OH: Battelle Memorial Institute Medical Research and Evaluation Facility, 2001); Roy Barnewall, \textit{Median Lethal Concentration (LC\textsubscript{50}) Determinations in Rhesus Monkeys Challenged with Different Strains of Bacillus anthracis Spores} (Columbus, OH: Battelle Memorial Institute Medical Research and Evaluation Facility, 2000); Claire Matthews, \textit{Anthrax LD\textsubscript{50} in Monkeys (Inhalation Exposure)} (Columbus, OH: Battelle Memorial Institute, 2001).


64 Henning et al., “Inhalational \textit{Bacillus anthracis} Exposure Therapeutic Model.”


spores all died, even though half were exposed to less than 10,000 spores.\textsuperscript{67} Combining both studies, the estimated inhaled doses are known for 21 African green monkeys.

In 2013, Savransky et al. published an alternative to the well-established non-human primate models, characterizing a guinea pig model of inhalation anthrax.\textsuperscript{68} The LD\textsubscript{50} was estimated to be 50,100 spores.

The control animals from various anthrax vaccine studies with rhesus macaques could also provide additional data points that could potentially be included in the calculation of infectivity or lethality parameters. In a 1993 study, one of ten monkeys survived challenge, but only the mean and standard deviation are given for the inhaled doses (400,000 ± 160,000 spores).\textsuperscript{69} Similarly, the control animals in a number of other studies were reported to have died following lethal exposures, which are expressed as summary statistics rather than individual doses.\textsuperscript{70} In contrast, the two controls for one study both died after exposures to specified amounts of agent (511 and 535 LD\textsubscript{50}, where LD\textsubscript{50} = 5.5x10\textsuperscript{4} spores).\textsuperscript{71} In a study published in 1963, 28 rhesus macaques were exposed to aerosolized \textit{B. anthracis}, and all but two died.\textsuperscript{72} The estimated doses for the two surviving monkeys were 10,100 and 10,400 spores, but the doses for the other monkeys were given in ranges.

d. Human Response Models

In a 2011 article,\textsuperscript{73} Day et al. describe an alternative method for calculating the probability of death due to infection with anthrax. It is based on a two-compartment mathematical model and is dose-dependent and considers the timing of antibiotic intervention. This model is substantially


different from the current infection and lethality models in $AMedP$-$8(C)$ in that it results in a nonzero fraction of the population becoming ill and surviving without treatment. Like the $AMedP$-$8(C)$ treatment model, the Day model allows for treatment at various times. The duration of illness is not an explicit output of the model, although the authors state that it could be used to provide an approximate estimate of the time to death. It may be worth comparing the results of this model with the existing $AMedP$-$8(C)$ model, which is dose-independent.

Egan et al.’s 2010 article identified a similar within-host model for calculating the probability of infection given an inhaled dose of anthrax and considers the effects of antibiotic prophylaxis.\(^7^4\) Again, it may be worthwhile to compare the results of this model with those of the $AMedP$-$8(C)$ infectivity model. The Egan article also reports levels of adherence with taking antibiotics, which may be useful to include in a treatment model for anthrax rather than assuming complete adherence to a prolonged antibiotic regimen. Another article also provides anthrax antibiotic adherence data collected via a survey of citizens across the country and specific areas affected by the 2001 anthrax attacks.\(^7^5\)

2. **Botulism**

   **a. Human Cases**

   From 2009 through 2012, more than 550 U.S. cases of botulism were reported to the CDC, categorized as infant (70%), foodborne (12%), or wound/unspecified (18%) botulism.\(^7^6\) Among the wound botulism cases were a number who contracted the disease via contaminated heroin, similar to the injection anthrax cases in Europe. Historically, this has been a problem in California,\(^7^7\) but recently there have also been cases reported in the states of Texas\(^7^8\) and Washington.\(^7^9\) No cases of inhalational botulism were identified in this literature review.

   **b. Medical Countermeasures**

   There is only one FDA-approved countermeasure for botulism in adults: BAT, a heptavalent antitoxin effective in neutralizing all seven known botulinum toxin serotypes (A, B,

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\(^7^6\) “Notifiable Diseases and Mortality Tables.”


\(^7^8\) ProMED-mail, “Botulism, Wound, Drug-Related—USA: (TX)” (ISID, 2011).

\(^7^9\) ProMED-mail, “Botulism, Wound, Drug-Related—USA: (WA)” (ISID, 2010); ProMED-mail, “Botulism, Wound, Drug-Related—USA (02): (WA)” (ISID, 2011).
C, D, E, F, and G).\textsuperscript{80} BAT, which is stockpiled in the SNS,\textsuperscript{81} replaced the licensed bivalent (A/B) antitoxin and the investigational serotype E antitoxin in March 2010.\textsuperscript{82} Still, the development of a vaccine to prevent botulism is ongoing, with multiple recombinant botulinum vaccine A/B candidates undergoing Phase II trials.\textsuperscript{83} Another product, a drug known as Firdapse (3,4-diaminopyridine), has also undergone Phase II and III clinical trials to treat patients with botulism in a hospital in France.\textsuperscript{84}

c. Animal Models

In 2010 the findings of a study to determine the LD\textsubscript{50} and LC\textsubscript{50} for inhaled botulinum toxin (serotypes A and B) in rhesus macaques was published as part of the process of establishing an appropriate animal model for inhalational botulism in compliance with the FDA Animal Rule for validating therapeutics for use in humans.\textsuperscript{85} In all, 40 monkeys were exposed (18 to serotype A and 22 to serotype B) and the median lethal values were established via probit analysis. The actual inhaled doses could not be estimated for 4 of the 18 monkeys exposed to serotype A (the specific threat modeled in AMedP-8(C)), so the dose-response data include only 14 data points.


(which are tabulated in the online supplement to the study). From these data, the authors reported an LD$_{50}$ of 550 MIPLD$_{50}$/kg for serotype A, which is higher than the 350 MIPLD$_{50}$/kg estimate currently used in $A_{\text{MedP}}$-8(C). [MIPLD is the mouse intraperitoneal lethal dose.] The study also defines the conversion of MIPLD$_{50}$ to grams as 3.2$\times$10$^{10}$ MIPLD$_{50}$/g (compared to 3.0$\times$10$^{10}$ MIPLD$_{50}$/g currently used as the conversion in $A_{\text{MedP}}$-8(C)). If these values were used directly in $A_{\text{MedP}}$-8(C) under the same assumption of a 70 kg man, the LD$_{50}$ would change from 0.8 μg/man to 1.2 μg/man. The report also lists the range of times to death for the eight animals (of the 18) that died, although the specific times for each animal are not provided. On the contrary, for the 22 monkeys exposed to serotype B, additional information (e.g., time to death for each animal) is captured in a second study published in 2011.

3. Brucellosis

a. Human Cases

Brucellosis, which is still endemic in large parts of the world, poses a significant public health risk, with an estimated 500,000 cases globally each year. According to a 2012 report, annual incident rates were estimated to be as high as 268 per 100,000 persons in some regions of the world. In the United States, brucellosis is relatively rare, with the annual number of cases averaging slightly more than 100 since 2009. By way of comparison, China reported approximately 26,000 annual cases of human brucellosis from 2005–2010.

b. Medical Countermeasures

Despite the global prevalence of brucellosis, there is still no vaccine licensed for use in humans. Live, attenuated vaccines have been used in the past on humans in the former Soviet

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90 “Notifiable Diseases and Mortality Tables.”
Union and in China, but their questionable efficacy and adverse side effects have precluded their use in other parts of the world.\textsuperscript{93} The current treatment of brucellosis in humans is a regimen of two or more antibiotics, although a number of studies have shown that the choice of antibiotics (among those commonly used) does not have a significant impact on the effectiveness of treatment.\textsuperscript{94} In past cases of brucellosis complicated by endocarditis, surgery in addition to antibiotic medical care corresponded to improved outcome.\textsuperscript{95}

c. Animal Models

As part of the process for acquiring FDA-approval for investigational vaccine candidates or other therapeutics under the Animal Rule, a research group has recently studied and validated both the mouse and rhesus macaque as appropriate models for testing therapeutics for inhalational brucellosis in humans.\textsuperscript{96} In the non-human primate study, 16 rhesus macaques were exposed to aerosolized \textit{B. melitensis} ranging from 5,440 to 511,000 CFU.\textsuperscript{97} All became infected (as measured by bacteria in at least one tissue at the time of euthanasia), and at least 15 of the 16 were febrile at some point during the course of illness. The pathology of brucellosis in rhesus macaques was well characterized, and the study supports the suitability of this animal as a model to test therapeutics under the Animal Rule.

A 2011 article described how to combine dose-response data from multiple species using published brucellosis experimental data.\textsuperscript{98} Not only were the experimental hosts different (mice, monkeys, and humans), but the routes of exposure differed as well. Using this method of pooling data from different studies, a beta-Poisson dose-response model was developed with an alpha value of 0.214149 and an $N_{50}$ (median infective dose) of 1,885 CFU. This technique may be useful for not only brucellosis, but also other agent models in the \textit{AMedP-8(C)} methodology. As this approach of combining dose-response data from multiple species and routes of exposure is in contrast to the hierarchy of data sources described in \textit{AMedP-8(C)}, a comparison of the two approaches is worthwhile. If the data pooling method is found to be more suitable for estimating

\begin{thebibliography}{98}
\bibitem{95} Keshtkar-Jahromi et al., “Treatment for \textit{Brucella} Endocarditis.”
\bibitem{97} Henning et al., “Rhesus Macaque Model for Inhalational Brucellosis.”
\end{thebibliography}
human response than choosing data from a single representative species and route of exposure, then most of the parameters in the \textit{AMedP-8(C)} human response models would need to be revisited for possible inclusion of additional data sources.

4. Glanders

a. Human Cases

Glanders in humans is rare, and the literature review of documents published since 2009 resulted in no cases of human glanders. Much of the current research on glanders and its causative agent, \textit{Burkholderia mallei}, is motivated by the pursuit of medical countermeasures. There is currently no licensed vaccine for preventing glanders in humans,\textsuperscript{99} and antibiotic therapy is the only treatment available.\textsuperscript{100}

b. Medical Countermeasures

A vaccine for glanders is still very early in the development cycle, and multiple approaches are being investigated, including live attenuated vaccines, subunit vaccines, and killed bacteria vaccines.\textsuperscript{101} Major challenges remain, including validating appropriate animal models for future FDA approval and ensuring additional protection against melioidosis, a similar disease caused by a related \textit{Burkholderia} species.\textsuperscript{102}

Little is known about the efficacy of antibiotic treatment of glanders, but \textit{B. mallei} is known to be resistant to many antimicrobials.\textsuperscript{103} Nevertheless, there are a number of antibiotics to which the bacterium is susceptible,\textsuperscript{104} and prolonged therapy with a combination of these drugs is recommended.\textsuperscript{105} Experimental therapy with monoclonal antibodies against \textit{Burkholderia mallei} and \textit{pseudomallei} is also under consideration.\textsuperscript{106}

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\textsuperscript{100} Silva and Dow, “\textit{Burkholderia mallei} and \textit{pseudomallei} Vaccines.”

\textsuperscript{101} Ibid.

\textsuperscript{102} Wolfe, Florence, and Bryant, “Biodefense Vaccine Programs and Challenges”; Choh et al., “\textit{Burkholderia} Vaccines.”


\textsuperscript{105} Estes et al., “Therapeutic Strategies for Melioidosis and Glanders.”
species has been tested in mice, and it was found that the most efficacious antibodies were those targeting the capsule of the bacteria.\textsuperscript{106}

c. Animal Models

Currently, large animal models (goats and non-human primates) of \textit{Burkholderia} species are limited to melioidosis, and their applicability to glanders is unclear.\textsuperscript{107} In the absence of other alternatives, these animal models should be investigated further and compared to the existing glanders human response parameter sources.

5. Plague

a. Human Cases

Seventeen cases of plague have been reported in the United States since 2009.\textsuperscript{108} The majority of cases were in rural parts of the western United States\textsuperscript{109} with the notable exception of a fatal laboratory-acquired case in Chicago, in which plague was somehow contracted from an attenuated strain of \textit{Y. pestis}.\textsuperscript{110} Cases of plague were also reported throughout the world, including Bolivia,\textsuperscript{111} China,\textsuperscript{112} Democratic Republic of Congo,\textsuperscript{113} Libya,\textsuperscript{114} Madagascar,\textsuperscript{115} Mongolia,\textsuperscript{116} Myanmar,\textsuperscript{117} Peru,\textsuperscript{118} Tanzania,\textsuperscript{119} and Uganda.\textsuperscript{120}

\textsuperscript{106} Shimin Zhang et al., “\textit{In Vitro} and \textit{in Vivo} Studies of Monoclonal Antibodies with Prominent Bactericidal Activity against \textit{Burkholderia pseudomallei} and \textit{Burkholderia mallei},” \textit{Clinical and Vaccine Immunology} 18, no. 5 (2011): 825–834; Estes et al., “Therapeutic Strategies for Melioidosis and Glanders.”


\textsuperscript{108} “Notifiable Diseases and Mortality Tables.”


\textsuperscript{110} ProMED-mail, “Plague, Fatal—USA: (IL), 2009, Lab Strain, CDC” (ISID, 2011); CDC, “Fatal Laboratory-Acquired Infection with an Attenuated \textit{Yersinia pestis} Strain—Chicago, Illinois, 2009,” \textit{MMWR} 60, no. 7 (2011): 201–205; ProMED-mail, “Plague, Fatal—USA (05): (IL) Lab Strain Susp. RFI” (ISID, 2009); ProMED-mail, “Plague, Fatal—USA (04): (IL) Lab Strain Susp. RFI” (ISID, 2009).

\textsuperscript{111} ProMED-mail, “Plague—South America: Bolivia, Peru, PAHO Report” (ISID, 2010); ProMED-mail, “Plague—Bolivia: (LP), Bubonic” (ISID, 2010).

\textsuperscript{112} ProMED-mail, “Plague, Pneumonic, 2009—China: (QH) Follow Up” (ISID, 2011); H. Wang et al., “A Dog-Associated Primary Pneumonic Plague in Qinghai Province, China,” \textit{Clin Infect Dis} 52, no. 2 (2011): 185–190; ProMED-mail, “Plague, Pneumonic—China (06): (QH), Who” (ISID, 2009); ProMED-mail, “Plague, Pneumonic—China (05): (QH) Comment” (ISID, 2009); ProMED-mail, “Plague, Pneumonic—China (04): (QH)” (ISID, 2009); ProMED-mail, “Plague, Pneumonic—China (03): (QH)” (ISID, 2009); ProMED-mail, “Plague, Pneumonic—China (02): (QH)” (ISID, 2009); ProMED-mail, “Plague, Pneumonic—China: (QH), RFI” (ISID, 2009); ProMED-mail, “Plague, Bubonic, Fatal—China: (GS)” (ISID, 2010); ProMED-mail, “Plague, Pneumonic—China: (Tibet Autonomous Region)” (ISID, 2010).
b. Medical Countermeasures

A human plague vaccine manufactured by Greer Laboratories, Inc. was licensed and used in the United States until 1999. Since the expiration of stored vaccines soon thereafter, a plague vaccine has not been available in the United States, but next-generation candidate vaccines are in development. In particular, F1 and V subunit vaccines have shown efficacy against both bubonic and pneumonic plague in non-human primate models, and at least one plague rF1V vaccine candidate has undergone Phase II clinical trials. Among the next steps in

113 ProMED-mail, “Plague—Congo DR: (OR)” (ISID, 2009).
114 ProMED-mail, “Plague, Bubonic—Libya (BN)” (ISID, 2011); ProMED-mail, “Plague, Bubonic—Libya (02): (BN)” (ISID, 2009); ProMED-mail, “Plague, Bubonic—Libya (BN)” (ISID, 2009).
115 ProMED-mail, “Plague—Madagascar (02): Fatalities” (ISID, 2012); ProMED-mail, “Plague—Madagascar: Fatalities” (ISID, 2012); ProMED-mail, “Plague, Pneumonic—Madagascar (05): (AV)” (ISID, 2011); ProMED-mail, “Plague, Pneumonic—Madagascar (04)” (ISID, 2011); ProMED-mail, “Plague, Pneumonic—Madagascar (03)” (ISID, 2011); ProMED-mail, “Plague, Pneumonic—Madagascar (02): (AS) Institut Pasteur Report” (ISID, 2011); ProMED-mail, “Plague, Pneumonic—Madagascar: (Antsiranana) RFI” (ISID, 2011).
116 ProMED-mail, “Plague, Pneumonic—Mongolia: (BO), RFI” (ISID, 2009).
117 ProMED-mail, “Plague—Myanmar: (YA)” (ISID, 2010).
118 ProMED-mail, “Plague—South America: Bolivia, Peru, PAHO Report”; ProMED-mail, “Plague – Bolivia: (LP), Bubonic”; ProMED-mail, “Plague—Peru (04): (LL)” (ISID, 2010); ProMED-mail, “Plague – Peru (03): (LL), PAHO” (ISID, 2010); ProMED-mail, “Plague – Peru (02): (LL), Bubonic, Pneumonic” (ISID, 2010); ProMED-mail, “Plague, Pneumonic—Peru: (TJ)” (ISID, 2010); ProMED-mail, “Plague, Bubonic – Peru: (LL), RFI” (ISID, 2010).
119 ProMED-mail, “Plague—Tanzania: (MY), RFI” (ISID, 2010).
the vaccine development process is establishing the animal model correlates of protection in humans. 125

Recent studies have investigated a number of different antibiotics to treat plague. In April 2012, the antibiotic Levaquin (levofloxacin) was approved for the treatment and post-exposure prophylaxis of plague. 126 Approval was based on a study in which Levaquin was successful in treating 16 of 17 non-human primates following inhalation exposure and subsequent fever. 127 In the same month, the FDA Anti-Infective Drugs Advisory Committee heard arguments for the approval of ciprofloxacin to treat plague, 128 but no record of their decision could be found, and a clinical trial to compare the safety and efficacy of ciprofloxacin and doxycycline to treat plague in humans was continuing to recruit patients in September 2012. 129 Although Gentamicin is used to treat plague and has been evaluated in clinical trials, it is still not indicated for this use by the FDA. 130

c. Animal Models

As it has with research on other biological agents of interest, the FDA Animal Rule has brought about a number of studies intended to establish various animal models as representative of human disease and for testing therapeutics for FDA approval. Among the recently developed animal models is a cynomolgus macaque model for pneumonic plague. A 2008 article by researchers from the Lovelace Respiratory Research Institute (LRRI) reported an inhalation LD50 in cynomolgus macaques of 66 CFU, 131 and another study by Battelle Biomedical Research

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125 Williamson and Oyston, “Protecting against Plague”; E. D. Williamson, “The Role of Immune Correlates and Surrogate Markers in the Development of Vaccines and Immunotherapies for Plague,” Advances in Preventive Medicine 2012 (2012); Feodorova and Motin, “Plague Vaccines.”
128 Robert Johnson, “Treatment of Pneumonic Plague: Medical Utility of Ciprofloxacin” (presentation at the Anti-Infective Drugs Advisory Committee Meeting of the National Institute of Allergy and Infectious Diseases, Silver Spring, MD, 3–4 April 2012); Katherine Laessig, “Ciprofloxacin for Pneumonic Plague” (presentation at the Anti-Infective Drugs Advisory Committee Meeting of the National Institute of Allergy and Infectious Diseases, Silver Spring, MD, 3–4 April 2012); FDA, “The Efficacy of Ciprofloxacin for Treatment of Pneumonic Plague” (briefing package, the Anti-Infective Drugs Advisory Committee Meeting of the National Institute of Allergy and Infectious Diseases, Silver Spring, MD, 3–4 April 2012).
130 FDA, “African Green Monkey (Chlorocebus aethiops) Animal Model Development to Evaluate Treatment of Pneumonic Plague,” (briefing package, the Anti-Infective Drugs Advisory Committee Meeting of the National Institute of Allergy and Infectious Diseases, Silver Spring, MD, 3–4 April 2012); “Treatment and Diagnosis of Plague,” ClinicalTrials.gov, last modified 24 February 2009, http://clinicaltrials.gov/show/NCT00128466.
Center researchers determined the LD$_{50}$ to be 24 CFU.\textsuperscript{132} Earlier studies by the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) established an LD$_{50}$ of 400 CFU.\textsuperscript{133}

As summarized in an FDA report to the Anti-Infective Drugs Advisory Committee in 2012, there were also a number of studies to establish the LD$_{50}$ and determine the pathology of pneumonic plague in the African green monkey.\textsuperscript{134} According to the report, USAMRIID conducted a study to determine the LD$_{50}$ in this species in 1993, which was calculated to be 343 CFU\textsuperscript{135} (more commonly reported as 350 CFU\textsuperscript{136}). Pathology results for these animals and other unvaccinated controls were later published in 1996.\textsuperscript{137} The FDA report also cites four subsequent major studies on the pathology of pneumonic plague in African green monkeys by USAMRIID (June 2003), LRRI (April 2007 and published in 2011\textsuperscript{138}), and Battelle Biomedical Research Center (July 2007 and January 2009). Combined, these four studies exposed 36 monkeys to target doses of 100 times the LD$_{50}$, and all but two monkeys died.

Studies on the effectiveness of various vaccines and other therapeutics may also provide information on the infective or lethal dose, as unvaccinated controls occasionally survive supralethal doses. Some vaccine studies on non-human primate models have been summarized recently.\textsuperscript{139}

\begin{itemize}
\item \textsuperscript{133} Louise M. Pitt, “Nonhuman Primates as a Model for Pneumonic Plague,” in \textit{Public Workshop on Animal Models and Correlates of Protection for Plague Vaccines} (Gaithersburg, MD: FDA, 2004).
\item \textsuperscript{134} FDA, “African Green Monkey (\textit{Chlorocebus aethiops}) Animal Model Development to Evaluate Treatment of Pneumonic Plague.”
\item \textsuperscript{135} Pitt, “Nonhuman Primates as a Model for Pneumonic Plague”; Judy Hewitt, “African Green Monkey Model of Pneumonic Plague” (presentation at the Anti-Infective Drugs Advisory Committee Meeting of the National Institute of Allergy and Infectious Diseases, Silver Spring, MD, 3–4 April 2012).
\item \textsuperscript{137} K. J. Davis et al., “Pathology of Experimental Pneumonic Plague Produced by Fraction 1-Positive and Fraction 1-Negative \textit{Yersinia pestis} in African Green Monkeys (\textit{Cercopithecus aethiops}),” \textit{Archives of Pathology & Laboratory Medicine} 120, no. 2 (1996): 156–163.
\end{itemize}
6. Q Fever

a. Human Cases

Since 2009, the CDC reported an average of approximately 130 cases of Q fever in the United States each year. In that same period, ProMED reported human cases of Q fever in Australia, Brazil, Germany, Hungary, the Netherlands, Serbia, Spain, and the United States. In addition, the disease was identified as endemic in Iran, Denmark, and Serbia and as a recently recognized disease in Japan. A 2013 article also described Q fever outbreaks from 1982 to 2010 in four countries (Bulgaria, France, Germany, and the Netherlands). The largest of these outbreaks was in the Netherlands from 2007 to 2010, in which 4,026 people were reportedly ill with Q fever, and a hospitalization rate of approximately 20% was reported.

b. Medical Countermeasures

The only current human vaccine for Q fever, Q-VAX, has been available since 1989, but it is not approved for use outside of Australia. Nevertheless, during the recent Q fever outbreaks in the Netherlands, Q-VAX was used to vaccinate populations at high risk of

140 “Notifiable Diseases and Mortality Tables.”
141 ProMED-mail, “Q Fever—Australia: (NS)” (ISID, 2011).
142 ProMED-mail, “Q Fever—Brazil: (MG)” (ISID, 2013).
143 ProMED-mail, “Q Fever—Germany: (NW, HE) Human, Animal” (ISID, 2011).
144 ProMED-mail, “Q Fever—Hungary: (BA) RFI” (ISID, 2013).
146 ProMED-mail, “Q Fever—Serbia: (VO)” (ISID, 2012).
147 ProMED-mail, “Q Fever—Spain: (AN)” (ISID, 2013).
148 ProMED-mail, “Q Fever—USA: Raw Cow’s Milk, Ex Goat” (ISID, 2011).
154 Ibid.
156 Georgiev et al., “Q Fever in Humans.”
developing chronic Q fever, and nearly two out of three vaccinated individuals reported adverse reactions to the vaccination.\textsuperscript{157} Work on a vaccine in the United States has been ongoing, and a Phase 2 study evaluating the safety of an inactivated, freeze-dried vaccine was scheduled, but as of August 2012, the study had “suspended participant recruitment.”\textsuperscript{158}

The benefits of post-exposure prophylaxis following a known or suspected exposure to Q fever are not proven, so the CDC recommendation is to seek medical attention for any acute febrile illness developed within six weeks of exposure.\textsuperscript{159} Doxycycline is the treatment of choice for both acute and chronic Q fever: a two week regimen for acute disease and months to years for chronic symptoms.\textsuperscript{160} Doxycycline is also being evaluated as a treatment for Q fever fatigue syndrome (QFS) at Radboud University in the Netherlands.\textsuperscript{161}

A number of articles included information that might affect the Q fever fatality or duration of illness submodels. Treated and untreated fatality rates were reported for chronic Q fever\textsuperscript{162} and for vascular complications of acute Q fever.\textsuperscript{163} The authors of a 2009 article interviewed 54 individuals that developed Q fever in 2007 and reported that 50 of them took an absence from work or school ranging from 2 to 296 days with a median of 21 days. For the hospitalized subset of these individuals (29), the duration of hospitalization ranged from 1 to 42 days with a median of 6 days.\textsuperscript{164}

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7. Staphylococcal Enterotoxin B (SEB)

a. Human Cases

Staphylococcal enterotoxin B (SEB), often associated with food poisoning, can result in toxic shock syndrome when delivered through a nonenteric route.\(^{165}\) Approximately 75 cases of staphylococcal toxic-shock syndrome have been reported to the U.S. CDC each year since 2009.\(^{166}\) Typically half of these cases are menstrual-related, and the rest are caused by other factors such as skin infections, burns, and post-surgery complications.\(^{167}\) No reports of aerosolized SEB exposure were discovered in this literature review.

b. Medical Countermeasures

Current therapy for SEB-induced toxic shock is mostly supportive care, although intravenous immunoglobulins may be effective when administered shortly after exposure.\(^{168}\) Pre-clinical tests indicate that other therapies may also be effective in treating toxic shock syndrome. Intranasal rapamycin, an immunosuppressive drug used to prevent graft rejection, was shown to protect mice from SEB-induced toxic shock as late as 17 hours after SEB exposure.\(^{169}\) Myeloid differentiation protein 88, MyD88, also shows promise in protecting against toxic shock syndrome caused by SEB.\(^{170}\) Anti-SEB human monoclonal antibodies have been found to neutralize toxin \textit{in vitro}\(^{171}\) and \textit{in vivo} in mouse models.\(^{172}\) Other studies indicated that

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166 “Notifiable Diseases and Mortality Tables.”


169 Krakauer and Buckley, “Intranasal Rapamycin Rescues Mice.”


combinations of different antibodies were more effective than a single antibody administered alone,\textsuperscript{173} and the addition of lovastatin further increased the efficacy of treatment in one study.\textsuperscript{174}

A number of SEB vaccine candidates are also under development. The recombinant vaccine STEBVax, perhaps the most advanced candidate, has been shown to be efficacious in mice and non-human primates\textsuperscript{175} and is currently recruiting volunteers for a Phase 1 human trial.\textsuperscript{176} A soybean-derived vaccine using the same nontoxic mutant form of SEB expressed as recombinant protein in \textit{E. coli} in the STEBVax vaccine was found to be as effective as the STEBVax vaccine in a piglet model.\textsuperscript{177} An oral formulation of the STEBVax vaccine also produced an antibody response against SEB in piglets.\textsuperscript{178}

8. \textbf{Smallpox}

\textbf{a. Human Cases}

In 1979, the World Health Organization certified the global eradication of smallpox.\textsuperscript{179} Yet more than 30 years later, smallpox is still considered a potential threat to public health, and research is ongoing to prevent and treat the disease.\textsuperscript{180} Developing vaccines and therapeutics in the absence of human disease presents a challenge, which is exacerbated by the fact that humans are the only known reservoir for the smallpox virus (orthopoxvirus variola), and no single animal model is capable of perfectly modeling smallpox in humans.\textsuperscript{181}

\begin{itemize}
  \item Tilahun et al., “Chimeric Anti-Staphylococcal Enterotoxin B Antibodies.”
  \item “Phase I STEBVax in Healthy Adults,” ClinicalTrials.gov, last modified 31 October 2013, \url{http://clinicaltrials.gov/ct2/show/NCT00974935}.
  \item Hudson et al., “Sublethal Staphylococcal Enterotoxin B Challenge Model.”
\end{itemize}
b. Medical Countermeasures

1) Vaccines

The eradication of smallpox throughout the world was due mainly to the extensive vaccination program. The live vaccinia virus vaccines used at that time, now referred to as first-generation vaccines, included Dryvax, Aventis Pasteur Smallpox Vaccine (APSV), and Lancy-Vaxina. The U.S. SNS currently contains more than 300 million doses of a second-generation smallpox vaccine, ACAM2000, which has immunogenicity and safety similar to Dryvax. They were derived from the same vaccinia strain, but ACAM2000 is manufactured using more modern cell culture technology. Another cell-culture derived smallpox vaccine, CJ-50300, has undergone clinical trials and was licensed by the Korean FDA in 2008. Because ACAM2000 can have serious side effects, third-generation vaccines are being developed using two immunogenic vaccinia strains that produce comparatively milder skin lesions, LC16m8 (licensed in Japan) and modified vaccinia Ankara (MVA). Three MVA vaccines have been tested in humans: TBC-MVA, ACAM3000, and MVA-BN (Imvamune). By the end of 2013, 20 million Imvamune vaccines will be available in the U.S. SNS for those unable to be vaccinated with the ACAM2000 vaccine. Although the Imvamune vaccine is not

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186 Moss, “Smallpox Vaccines.”


licensed, in the case of a smallpox outbreak, the vaccine could be made available under an IND protocol.

Genetic engineering allows for the deletion of various genes with the aim of attenuating the virus without sacrificing immunogenicity. Investigational fourth-generation vaccine candidates that leverage genetic engineering include NYVAC, defective vaccinia virus Lister (dVV-L), and VACVDE3L. In addition to these, a dual vaccine, protective against smallpox and anthrax, has been tested on both mice and rabbits.

2) Therapeutics

Vaccinia immune globulin (VIG) is the only FDA approved product for treating complications from smallpox vaccinations, and it is now available in both intramuscular (IM) and IV forms. There is also evidence that VIG administered as a post-exposure prophylaxis along with vaccination, reduces the incidence of smallpox in humans. A secondary treatment for adverse effects of smallpox vaccination that could be used under the FDA IND protocols is cidofovir, which has also proven effective as a post-exposure prophylaxis in rabbitpox model. In another study, single-dose cidofovir treatments protected mice after exposure with ectromelia (mousepox) virus. Monoclonal antibodies have also shown promise in animal models, and a cocktail of monoclonal antibodies could potentially enhance the efficacy of VIG or even replace it in the future.

189 Henderson, “Smallpox Virus Destruction.”
193 Merkel et al., “Dual Vaccine against Smallpox and Anthrax.”
195 Xiao and Isaacs, “Treatment of Orthopoxvirus Infections.”
196 “Smallpox (Vaccinia) Vaccine Adverse Reactions.”
199 Xiao and Isaacs, “Treatment of Orthopoxvirus Infections.”
Encouraging results from animal experiments have prompted human clinical trials on two antiviral drugs, CMX001 and ST-246. CMX001 was shown to prevent lethality in rabbits when used as a pre- and post-exposure prophylaxis against rabbitpox virus, although symptoms of disease were still manifest. It also afforded protection as a treatment once symptoms appeared, although the effectiveness decreased the longer treatment was delayed. Likewise, ST-246 shows potential as a prophylactic measure and treatment against aerosol exposure of orthopoxviruses and, surprisingly, may also provide additional benefits when given in combination with vaccination.

c. Animal Models

A recent study administering the variola virus intravenously to cynomolgus macaques found that the macaque model is “an excellent surrogate for human smallpox in terms of disease onset, acute disease course, and gross and histopathological lesions.” Yet the IV route of exposure may limit the usefulness of this model for approving smallpox therapeutics in humans, and attempts at validating an aerosol model of variola virus in non-human primates were not successful.

Since most animals are naturally resistant to the variola virus, related orthopoxviruses that approximate human smallpox in various animal species have been investigated, making it possible to extrapolate the effects of vaccine and antiviral candidates. Among these are the calpox virus in marmosets, rabbitpox virus in rabbits, cowpox virus in non-human

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202 Ibid.


primates, monkeypox virus in non-human primates and prairie dogs, and vaccinia and ectromelia virus in mice.

9. Tularemia

a. Human Cases

Tularemia is generally believed to be a disease unique to the northern hemisphere, but there are recent reports of women in the Australian state of Tasmania contracting tularemia after being scratched or bitten by possums in 2011. Nevertheless, the disease is endemic in North America, Europe, and Asia, as reflected by ProMED reports of recent cases diagnosed in Canada, Germany (likely contracted in Turkey), Norway, Russia, and Turkey.


214 ProMED-mail, “Tularemia, Pneumonic—Canada: (AB) Biologic Immunomodulator” (ISID, 2012).

215 ProMED-mail, “Tularemia, Imported—Germany: (Berlin) Ex Turkey, Alert” (ISID, 2011).

216 ProMED-mail, “Tularemia—Norway (02): (Central)” (ISID, 2011).

217 ProMED-mail, “Tularemia—Russia (02): (KM)” (ISID, 2013).

Tularemia is also endemic in the United States, with an average of more than 130 cases of tularemia reported to the CDC annually from 2009 to 2012. Of the 190 tularemia cases in Missouri reported from 2000 to 2007, clinical records were available for 121 and were summarized in two articles. The reports documented the incubation periods (ranging from one to nine days with a median of three days) and clinical forms of the disease (including 26 pneumonic cases with six known inhalational exposures), but the comprehensive dataset specifying incubation periods for pneumonic tularemia patients was not published. Since the combined data are not available and the exposures were not of known doses, these data are less useful for developing an incubation period submodel than the human exposure data from the tularemia MRV experiments currently used in AMedP-8(C).

b. Medical Countermeasures

While a number of antibiotics have been proven effective in treating tularemia, there is still no tularemia vaccine licensed for general use in the United States. A live vaccine strain (LVS), developed by the Soviet Union and gifted to the United States in 1956, at one point had IND status. Yet today the vaccine is used only for at-risk military and laboratory personnel. The LVS vaccine has proven to be effective in reducing the incidence of laboratory-acquired tularemia, has protected nonhuman primates challenged with high aerosol doses, and has

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219 “Notifiable Diseases and Mortality Tables.”
222 Weber et al., “Clinical Recognition and Management of Tularemia.”
228 Barry, Cole, and Santiago, “Vaccines against Tularemia.”
undergone human clinical studies. Nevertheless, the fact that the LVS vaccine is based on an attenuated Type B strain of *F. tularensis* and only partially protects against virulent Type A challenge, among other drawbacks, has led to further investigation into alternative tularemia vaccines. A number of vaccine candidates (acellular subunit, killed whole cell, and live attenuated vaccines) have been developed and tested in mice, and two are also being tested in nonhuman primate models at LRRI. Studies indicate that respiratory vaccination may be the best protector against aerosol challenge.

c. Animal Models

Nonhuman primate studies on tularemia have been conducted on at least three species. Investigations with rhesus macaques were conducted in the 1960s and 1970s, and more recently models have been validated in African green monkeys (at USAMRIID) and cynomolgus macaques (at LRRI). The cynomolgus macaque LD<sub>50</sub> for tularemia was 1–2 CFU, and a relationship between dose and time to death was found in this species, which may be useful to developing a dose-dependent time-to-death model for humans. No such relationship was found in the five African green monkeys exposed at USAMRIID.

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231 Barry, Cole, and Santiago, “Vaccines against Tularemia.”


233 Sherwood, “Protecting Our Country.”

234 Pechous, McCarthy, and Zahrt, “*Francisella tularensis* Pathogenesis.”


237 Valderas et al., “Determination of the *Francisella tularensis* SCHU S4 Aerosol LD<sub>50</sub> in Cynomolgus Macaques and Characterization of Tularemia Manifestation.”

238 Twenhafel, Alves, and Purcell, “Pathology of Inhalational *Francisella tularensis*.”
10. Venezuelan Equine Encephalitis (VEE)

a. Human Cases

Since 2009, ProMED has reported cases of Venezuelan equine encephalitis (VEE) in Belize,\(^ {239}\) Panama,\(^ {240}\) and Venezuela\(^ {241}\) and one suspected case in Columbia.\(^ {242}\) Although VEE is not on the U.S. CDC list of notifiable diseases,\(^ {243}\) there was no indication on PubMed or the CDC website that there were any recent cases of VEE in the United States. The CDC does, however, have recorded cases of the related alphaviruses eastern equine encephalitis (EEE) and western equine encephalitis (WEE); since the beginning of 2009, there were 36 EEE cases and no WEE cases reported.\(^ {244}\)

b. Medical Countermeasures

Although there are currently no VEE vaccines or antiviral drugs that are licensed for use in humans,\(^ {245}\) a live-attenuated vaccine, TC-83, and a formalin-inactivated variant of TC-83, C-84, have been used for decades in the United States to protect laboratory workers and other at-risk personnel under IND protocols.\(^ {246}\) Due to concerns over the safety and immunogenicity of these vaccines, there has been considerable effort to develop next generation vaccines.\(^ {247}\)

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240 ProMED-mail, “Venezuelan Equine Encephalitis—Panama (03): (DR) Fatal” (ISID, 2010); ProMED-mail, “Venezuelan Equine Encephalitis—Panama (02): (DR) Fatal” (ISID, 2010); ProMED-mail, “Venezuelan Equine Encephalitis—Panama: (DR) Fatal” (ISID, 2010).

241 ProMED-mail, “Venezuelan Equine Encephalitis—Venezuela: Humans, Equines” (ISID, 2010).


244 “Notifiable Diseases and Mortality Tables.”


Among the various vaccine candidates are live-attenuated vaccines such as V3526, which proved efficacious in animals but caused adverse effects in Phase 1 human clinical trials.\(^{248}\) Although attenuated vaccines are typically highly immunogenic, most rely on serial passage of a virulent virus strain through a culture medium (83 passages for TC-83),\(^{249}\) which introduces a few point mutations that could potentially revert back to the virulent strain upon virus replication.\(^{250}\) To overcome these shortcomings, several vaccine candidates have been developed that combine VEE virus structures with those from other viruses, such as Sindbis virus chimeric,\(^{251}\) encephalomyocarditis virus,\(^{252}\) and adenoviruses.\(^{253}\) In addition, formalin-inactivated and gamma irradiation-inactivated versions of V3526 (fV3526 and gV3526, respectively) have been tested in mouse models and shown to be at least as efficacious as C-84.\(^{254}\) Other vaccine candidates include DNA vaccines\(^{255}\) and pseudoinfectious virus (PIV) vaccines.\(^{256}\)

In addition to vaccine research, other areas of medical countermeasure development have seen progress. Most notably, mouse monoclonal antibodies have been shown to protect mice from aerosol and subcutaneous challenge with VEE virus, although the antibodies would need to be “humanized” before being used in humans.\(^{257}\)

\(^{248}\) Fine et al., “Development and Evaluation of Inactivated Vaccines”; Williams et al., “Gene Optimised Adenovirus-Based Vaccine.”

\(^{249}\) Dupuy et al., “Efficacy of a DNA Vaccine.”


\(^{251}\) Rossi et al., “IRES-Based Venezuelan Equine Encephalitis Vaccine”; Dupuy et al., “Efficacy of a DNA Vaccine.”

\(^{252}\) Rossi et al., “IRES-Based Venezuelan Equine Encephalitis Vaccine.”


\(^{256}\) Atasheva et al., “Pseudoinfectious Venezuelan Equine Encephalitis Virus.”

C. Chemical Agents

1. Distilled Mustard (HD)

   a. Human Cases

   While the United States nears completion of the destruction of its chemical weapons stockpiles, elsewhere in the world, chemical agents are still a battlefield threat. The Syrian military, for instance, is known to have stockpiles of chemical agents, including HD, and both sides in the ongoing civil war have confirmed the use of chemical nerve agents.

   Although chemical agent attacks with HD have not been reported in Syria, other recent exposures to HD have been confirmed. In March 2013, it was reported that 20 guards in Libya were exposed to HD while securing a chemical weapons storage facility. The guards were transported to Europe for specialized treatment, but the extent of their injuries is unknown.

   In 2010 and 2012, U.S. commercial fishermen dredging for clams discovered discarded munitions filled with HD. In the 2010 incident, one of the fishermen was admitted to the hospital for five days and had multiple lesions on his skin, while another man was evaluated and released. In the 2012 episode, none of the potentially exposed individuals developed symptoms of mustard poisoning.

   In addition to these more recent experiences, the literature review revealed a number of review articles that summarized the physiological effects of HD exposure and the current knowledge of its mechanisms of action and potential treatment options. In addition, several

263 Fendick et al., “Exposures to Discarded Sulfur Mustard Munitions.”
studies have investigated the long-term sequelae of HD exposure in military and civilian populations exposed in the Iran-Iraq War in the 1980s. The molecular pathogenesis of HD injury is incomplete, but the current knowledge is well summarized in these review articles.

b. Medical Countermeasures

Although there is no specific antidote for HD poisoning, a number of reports have identified potential therapeutics for cutaneous lesions resulting from HD exposure. One article categorized countermeasure approaches into six strategies: intracellular scavengers, DNA cell cycle modulators, PARP inhibitors, calcium modulators, protease inhibitors, and anti-inflammatory compounds. This article also identified 19 candidate countermeasures with greater than 50% efficacy in the mouse ear vesicant model, which fell into four of the six pharmacologic strategies mentioned above (all except DNA cell cycle modulators and calcium modulators). In addition, antioxidant therapies, iodine, and baicalin have reportedly demonstrated some therapeutic benefits in treating cutaneous mustard lesions.


2. Sarin (G) and Methylphosphonothioic Acid (VX)

a. Human Cases

Sarin and VX are nerve agents that were stockpiled by many countries during or after WWII.\textsuperscript{272} Sarin is the chemical or biological agent most recently reported to have been used in warfare. In June 2013, France, the United States, and the United Nations reported that the regime of Syrian President Bashar al-Assad used sarin in the Syrian civil war and was responsible for an estimated 100 to 150 nerve agent casualties.\textsuperscript{273} In a July 2013 statement, the Russian ambassador to the United Nations asserted that Syrian rebels were responsible for using sarin in an attack that killed at least 26 in March 2013.\textsuperscript{274}

b. Medical Countermeasures

The majority of the peer-reviewed literature related to sarin and VX focuses on therapeutic advancements. The standard treatment for nerve agent poisoning consists of atropine, an oxime (2-PAM in the United States), and an anticonvulsant (diazepam in the United States), but replacements or adjuncts for each of these components of therapy are being developed or tested. For instance, atropine combined with galantamine protected mice even when treatment was delayed until 30 to 45 minutes post-exposure.\textsuperscript{275} A number of new oximes are also under investigation. HI-6 and MMB-4 have been proposed as replacements for the currently fielded oxime 2-PAM.\textsuperscript{276} Other potential oximes of interest include scopolamine,\textsuperscript{277} TAB2OH,\textsuperscript{278}


\textsuperscript{276} Paul M. Lundy et al., “Comparative Protective Effects of HI-6 and MMB-4 against Organophosphorous Nerve Agent Poisoning,” Toxicology 285, no. 3 (2011): 90–96.

\textsuperscript{277} I. Koplovitz and S. Schulz, “Perspectives on the Use of Scopolamine as an Adjunct Treatment to Enhance Survival Following Organophosphorus Nerve Agent Poisoning,” Military Medicine 175, no. 11 (2010): 878–882.
K027,\textsuperscript{279} and K203.\textsuperscript{280} Tertiary oximes, such as monoisonitrosoacetone (MINA), diacetylmonoxime (DAM), and pro-2-PAM are capable of reactivating acetylcholinesterase in the central nervous system and are therefore more effective at preventing seizures than the quaternary oximes 2-PAM, HLö7, and MMB-4.\textsuperscript{281} In 2006, a Phase 1 trial was completed testing midazolam as a potential anticonvulsant replacement for diazepam.\textsuperscript{282}

For percutaneous VX, topical skin barrier creams can be used as a form of pretreatment to reduce the amount of agent absorbed. A number of such skin protectants have been tested \textit{in vitro} and some have begun safety testing in humans.\textsuperscript{283}

Experiments with bioscavengers as alternatives to traditional nerve agent treatment are also being reported. A human serum butyrylcholinesterase was tested in rats\textsuperscript{284} and completed Phase 1 human trials in 2008 for both IM and IV administration.\textsuperscript{285} In 2009, a recombinant human

\begin{footnotesize}
\begin{itemize}
\item “Phase I Trial for Intramuscular Administration of Midazolam Using an Autoinjector (AAS),” ClinicalTrials.gov, last modified 21 September 2007, \url{http://www.clinicaltrials.gov/ct2/show/NCT00534378}.
\end{itemize}
\end{footnotesize}
butyrlcholinesterase, Protexia, completed a Phase 1 clinical trial. Catalytic bioscavengers such as organophosphorus hydrolase (OPH) and paraoxonase 1 are also being investigated for protection against nerve agent toxicity.

Other forms of treatment include tropicamide, a topical anticholinergic drug, which was reported to decrease miosis without the side effects of atropine or homatropine (“mydriasis and partial cycloplegia, which may worsen visual performance”). Another article emphasized the need to consider delayed treatments, such as brain cell therapy, neuroregeneration, and cytokine cocktail treatment, to repair nerve agent-induced brain lesions.

D. Radiation

1. Human Cases

Perhaps the most noteworthy radiological event since 2009 was the Fukushima Daiichi nuclear disaster in Japan on 11 March 2011. Although large amounts of radioactive material were released from the power plant into the environment, nobody was reported to have received doses high enough to cause acute radiation syndrome (ARS). In contrast, other recent radiation accidents have resulted in symptoms and even death. In 2010, seven people were hospitalized after accidental exposure to $^{60}\text{Co}$ in a pile of scrap metal in India, one of whom died from his injuries within weeks. Later that same year, the U.S. FDA announced that it was aware of approximately 385 patients who were exposed to excess radiation (> 0.5 Gy) during CT brain perfusion scans in U.S. hospitals. The exposures were high enough to cause hair loss and redness of the skin in some patients.

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For the incidents mentioned above, doses and clinical symptom progressions were unavailable, but such information may be available for hundreds of cases in the System for Evaluation and Archiving of Radiation accidents based on Case Histories (SEARCH) database, a collection of 785 case histories from human cases of radiation exposure. With access to the SEARCH database, IDA could use its records to validate or revise the human response models in AMedP-8(C). The database is currently being leveraged by an international group of experts known as the Group to Link nonhuman Primate and Human radiation effects (GLiPH), which is working to combine human and non-human primate response data to improve the medical management of radiation casualties.

Other sources of radiation effects information that could be useful to the AMedP-8(C) effort to model the human response to radiation include dose-response models based on acute radiation accidents in Russia and a radiation effects database called FREDERICA, which contains 1,228 radiation exposure records for a variety of flora and fauna, including 269 mammal exposure records.

2. Medical Countermeasures

Radiation countermeasures can be grouped into three categories based on the timing of their administration. Drugs in the first category, administered prior to irradiation, are known as radioprotectants or radioprotectors. Experiments with dozens of candidate radioprotectants have been reported, but amifostine is the only one that is currently approved for use as a radioprotectant. Radiation mitigators make up the second class of countermeasures, and they are administered as post-exposure prophylaxis, before the onset of overt symptoms. The final

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295 Maidment et al., Group to Link Nonhuman Primate and Human Radiation Effects (GLiPH).


category of radiation countermeasures is therapeutic agents, which are used to treat symptoms of irradiation. Because a number of radiation mitigators are also used as therapeutic agents, all post-exposure radiation countermeasures will be discussed together.

A number of FDA-approved drugs can be used to mitigate the effects of radiation by reducing the amount of internal radiation that the body absorbs. Prussian blue, calcium diethylenetriamine pentaacetate (DTPA), zinc DTPA, and potassium iodide have different mechanisms of action, but all serve to expedite the passing of radioactive materials through the body so they have less time to cause damage.

Other radiation mitigators include growth factors such as granulocyte-colony stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF), which stimulate hematopoiesis. Hematopoiesis can also be induced through the IV administration of mesenchymal stem cells, mesenchymal stromal cells, myeloid progenitor cells, or hematopoietic stem cells. One expert panel concluded that there is strong evidence to support the use of G-CSF or GM-CSF and weak evidence to support hematopoietic stem cell transplantation to treat hematopoietic symptoms of ARS. The same expert group also reported recommendations for physiological systems other than the hematopoietic system.

Although currently there are no approved pharmaceuticals for ARS, G-CSF and four other drugs can be used under IND protocol. Genistein and 5-androstenediol have both been

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shown to be efficacious in mice.\textsuperscript{310} CBLB502 has also been tested in mouse and non-human primate models and shown efficacy in pre-exposure and post-exposure uses.\textsuperscript{311} In addition to being safe and effective as a radioprotectant in a mouse model (with a dose reduction factor of 1.16)\textsuperscript{312} and when administered after exposure,\textsuperscript{313} Ex-Rad (ON01201.Na) has also undergone preclinical safety experiments in other animal species including rats, rabbits, canines, and non-human primates.\textsuperscript{314}

3. Animal Models

As for chemical and biological countermeasures, approval for radiation countermeasures must sometimes rely on animal models, since human efficacy testing is unethical. A number of models that represent various sub-syndromes of ARS in humans have recently been developed in different animal species. Mouse and non-human primate models have been developed for gastrointestinal symptoms of ARS.\textsuperscript{315} Additionally, the hematopoietic sub-syndrome has been modeled in mice, minipigs, and non-human primates.\textsuperscript{316} Lastly, mice, rats, canines, pigs, and

\begin{itemize}
\item \textsuperscript{313} Shubhankar Suman et al., “Administration of ON 01201.Na after Exposure to Ionizing Radiation Protects Bone Marrow Cells by Attenuating DNA Damage Response,” \textit{Radiation Oncology} 7, no. 6 (2012).
non-human primates are all being developed as animal models for radiation-induced lung injuries.317

3. **Estimation of Effort Required to Extend**  
*AMedP-8 Methodology*

**A. Introduction**

The literature review revealed three categories of work that could be carried out to update or extend the AMedP-8(C) methodology: (1) editorial changes to the text of future versions of AMedP-8 or related documents, (2) the incorporation of new data into existing AMedP-8(C) models, and (3) the comparison of AMedP-8(C) models to other published models or databases for validation or revision. Estimates for the level of effort required to complete future analyses identified in this review were based on IDA’s prior experiences performing analyses in this field.

**B. Editorial Changes**

Some recent advances, such as the truncated primary dosing schedule for the anthrax vaccine BioThrax, represent important changes to the application of the medical management of CBRN casualties, but cause little modification to the AMedP-8(C) methodology. Other similar developments include the approval of another antibiotic, Levaquin (levofloxacin), as a plague post-exposure prophylaxis and treatment and the inclusion of Imvamune smallpox vaccines for individuals contraindicated for the ACAM2000 vaccine. Advances such as these may require some editorial revisions to outdated information in text or tables but will not require significant analysis by IDA researchers.

Incorporating changes of this kind into future versions of AMedP-8 or other related documents represents a relatively minor level of effort for IDA researchers. If done in isolation, this work would take an estimated one person-month of effort to rewrite, review, and publish. However, it is likely that these changes would be made as part of a larger effort to revamp the methodology (i.e., development of the next version of AMedP-8), and these changes would add an insubstantial amount of work to that effort.

**C. Incorporation of New Data into Existing Models**

The second category of changes involves incorporating known sets of data into the AMedP-8(C) methodology. As described in the sections above, animal and human response data have become available for a number of agents modeled in AMedP-8(C). In particular, potential changes were identified for a number of biological agent submodels: anthrax infectivity and lethality; botulism infectivity, lethality, and duration of illness; brucellosis infectivity; glanders infectivity; plague infectivity and lethality; Q fever duration of illness; smallpox infectivity...
(modified by potential post-exposure prophylactic administration of VIG); and tularemia duration of illness.

The process of extracting the latest data from published sources, fitting a distribution to the new combined data set, and documenting the results is estimated to take, on average, one-quarter person-month of effort per submodel. As there are 12 submodels that could change, this effort is estimated to take a total of approximately three person-months. Before any changes are made to the human response models in AMedP-8(C), a higher-level analysis should be conducted to determine whether new data would significantly impact the casualty estimates and improve utility for military planners. Such an analysis is estimated to require approximately one person-month.

In addition to the data related to the biological agents in AMedP-8(C), significant information is available on the medical countermeasures available to prevent, mitigate, or treat the effects of radiation exposure. The impact of radioprotectant drugs and radiation injury treatments on the casualty estimate should be a focus of future IDA analysis. A few publications that provided concrete efficacy data on the various countermeasures were gathered in this literature review, but more significant work is required to quantify their effects. For this reason, this analysis is estimated to require three person-months of effort.

A worthwhile related effort is a validation of the AMedP-8(C) radiation models using case histories from the SEARCH radiation effects database. A first step for IDA would be to gain access to the 785 case histories in the database. Although it is unlikely that all cases report estimated doses, it is possible that many cases can be used to revise or validate the AMedP-8(C) radiation models. This effort is estimated to require four person-months of effort.

IDA should also maintain an awareness of ongoing GLiPH efforts to combine human and non-human primate radiation response data, which leverages the SEARCH database. IDA should evaluate any pertinent work performed by the GLiPH team for possible incorporation into the AMedP-8(C) methodology, although the level of effort required to do so is impossible to estimate without a better understanding of the GLiPH team’s work.

D. Evaluation of Alternative Models

The third category of changes that could be made to the AMedP-8(C) methodology involves a higher-level assessment of the process and an evaluation of whether the human response models in AMedP-8(C) are the best models to use in light of recently published alternatives. Alternative dose-response models were identified for anthrax and radiation. In addition, a general dose-response method of pooling data from multiple species was described for both brucellosis and Q fever.318 This method, if appropriate, could be extended to combine data from multiple

species for every agent modeled in $AMedP-8(C)$ and would potentially replace the hierarchy of data quality used throughout the $AMedP-8(C)$ methodology.

Understanding and evaluating the utility of alternative methodologies is a significant effort that is estimated to require three person-months of effort. The level of effort for any potential follow-on work to revise the $AMedP-8(C)$ methodology to incorporate new models deemed more appropriate would have to be estimated at a later time.
4. **Recommended Future Analyses**

Based on IDA’s understanding of the available literature and the needs of the sponsor, the IDA research team recommends a number of future efforts related to *AMedP-8(C)* human response modeling. These include addressing editorial changes and past methodological advances (1), considering new data (2, 3, and 4), evaluating alternative models (5), and investigating outstanding topics recommended in prior annual reviews (6 and 7).

1. As a NATO document, *AMedP-8(C)* is subject to a periodic review every three years. Since its 2011 publication, the *AMedP-8(C)* methodology has been expanded to include human response parameters for additional agents and the consideration of medical care. Given these significant advancements, IDA recommends that a new version of *AMedP-8* be proposed at the 2014 review. The proposal should include incorporating, at a minimum, the new agents, the impact of medical care, and any editorial changes to keep the content current, as described in the previous section of this document.

2. During this review, the IDA team was successful in identifying new sources of data relevant to updating the *AMedP-8(C)* methodology. In particular, data are available that could impact the anthrax, botulism, brucellosis, glanders, plague, Q fever, smallpox, and tularemia models. In addition, IDA continues to pursue access to the human response studies conducted through the MRV program in the 1950s and 1960s, which could provide data useful to the Q fever, SEB, and tularemia models. IDA should conduct cost-benefit analyses to determine whether the new data would significantly improve the military medical planning process and warrant changes to the *AMedP-8(C)* methodology.

3. The IDA team should quantify the impact on the casualty estimate of radioprotectant drugs, radiation mitigators, and radiation therapeutic agents in NATO member national inventories or those in procurement, but not fielded. As some of these countermeasures are FDA-approved or have emergency use IND status, some efficacy data must be available.

4. Case histories from the SEARCH radiation effects database should be reviewed to assess their value in validating or revising the *AMedP-8(C)* radiological agent human response models. In addition to requesting access to the SEARCH database, IDA should reach out to and collaborate with the GLiPH team, which is leveraging the SEARCH data to establish correlations between human and non-human primate radiation exposures. With a better understanding of the GLiPH team’s efforts, IDA can determine how their work might fit within the framework of the *AMedP-8(C)* methodology.
5. The IDA team should compare the *AMedP-8(C)* dose-response models to the alternative dose-response models discovered in this literature review and any other published models. In particular, alternative dose-response models specific to anthrax and radiation were discovered, as well as a more general method of pooling infectivity data from multiple species. Analyses should be conducted to compare each alternative methodology with the existing models within *AMedP-8(C)*. The result of these analyses should be a recommendation to continue with the current methodology or to change it, along with an estimate of the level of effort required to do so.

6. Many chemical and biological agents of interest to various government agencies are candidates for future inclusion in *AMedP-8(C)*. Levels of effort to incorporate more than 40 agents into the *AMedP-8(C)* methodology were estimated in the 2009 review, yet only a small fraction has been modeled. IDA should develop a prioritization scheme for future inclusion of the remaining agents in *AMedP-8(C)* based on an analysis of the military threat or capability to NATO nations and the availability of modeling data for each agent.

7. As discussed in prior annual reviews, IDA stands ready to investigate the feasibility of incorporating the estimation of psychological casualties into the *AMedP-8(C)* methodology if and when this becomes a sponsor priority.
Appendix A

References


Henderson, D. A. “Smallpox Virus Destruction and the Implications of a New Vaccine.” 


Hewitt, Judy. “African Green Monkey Model of Pneumonic Plague.” Presentation at the Anti-Infective Drugs Advisory Committee Meeting of the National Institute of Allergy and Infectious Diseases, Silver Spring, MD, 3–4 April 2012.


Johnson, Robert. “Treatment of Pneumonic Plague: Medical Utility of Ciprofloxacin.” Presentation at the Anti-Infective Drugs Advisory Committee Meeting of the National Institute of Allergy and Infectious Diseases, Silver Spring, MD, 3–4 April 2012.


Oschewitz, Jon, Fen Yu, and Kemp B. Cease. “A Synthetic Peptide Vaccine Directed against the 2β2-2β3 Loop of Domain 2 of Protective Antigen Protects Rabbits from Inhalation Anthrax.” *Journal of Immunology* 185, no. 6 (15 Sep 2010): 3661–3668.


—. “Plague—Bolivia: (LP), Bubonic.” ISID, 2010.
—. “Plague—Congo DR: (OR).” ISID, 2009.
—. “Plague—Tanzania: (MY), RFI.” ISID, 2010.
—. “Plague, Bubonic—Libya: (BN).” ISID, 2011.
—. “Plague, Bubonic—Peru: (LL), RFI.” ISID, 2010.
—. “Plague, Fatal—USA: (IL), 2009, Lab Strain, CDC.” ISID, 2011.
—. “Plague, Pneumonic—Madagascar (03).” ISID, 2011.
—. “Plague, Pneumonic—Mongolia: (BO), RFI.” ISID, 2009.


Smith, William J. “Therapeutic Options to Treat Sulfur Mustard Poisoning—the Road Ahead.” *Toxicology* 263, no. 1 (1 Sep 2009): 70–73.


# Appendix B
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>AIG</td>
<td>Anthrax Immune Globulin</td>
</tr>
<tr>
<td>AMedP-8</td>
<td>Allied Medical Publication 8</td>
</tr>
<tr>
<td>AMedP-8(C)</td>
<td>Allied Medical Publication 8 (C)</td>
</tr>
<tr>
<td>APSV</td>
<td>Aventis Pasteur Smallpox Vaccine</td>
</tr>
<tr>
<td>ARS</td>
<td>Acute Radiation Syndrome</td>
</tr>
<tr>
<td>CBRN</td>
<td>Chemical, Biological, Radiological, and Nuclear</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
</tr>
<tr>
<td>CFU</td>
<td>Colony Forming Unit</td>
</tr>
<tr>
<td>CUD</td>
<td>Common User Database</td>
</tr>
<tr>
<td>DAM</td>
<td>Diacetylmonoxime</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>DOD</td>
<td>Department of Defense</td>
</tr>
<tr>
<td>DTPA</td>
<td>Diethylenetriamene Pentaacetate</td>
</tr>
<tr>
<td>dVVL</td>
<td>Defective Vaccinia Virus Lister</td>
</tr>
<tr>
<td>EEE</td>
<td>Eastern Equine Encephalitis</td>
</tr>
<tr>
<td>EF</td>
<td>Edema Factor</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>G-CSF</td>
<td>Granulocyte-Colony Stimulating Factor</td>
</tr>
<tr>
<td>GB</td>
<td>Sarin</td>
</tr>
<tr>
<td>GLiPH</td>
<td>Group to Link Nonhuman Primate and Human Radiation Effects</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>Granulocyte-Macrophage Colony-Stimulating Factor</td>
</tr>
<tr>
<td>HD</td>
<td>Distilled Mustard</td>
</tr>
<tr>
<td>IDA</td>
<td>Institute for Defense Analyses</td>
</tr>
<tr>
<td>IM</td>
<td>Intramuscular</td>
</tr>
<tr>
<td>IND</td>
<td>Investigational New Drug</td>
</tr>
<tr>
<td>ISID</td>
<td>International Society for Infectious Diseases</td>
</tr>
<tr>
<td>IV</td>
<td>Intravenous</td>
</tr>
<tr>
<td>KBMA</td>
<td>Killed But Metabolically Active</td>
</tr>
<tr>
<td>LC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Median Lethal Concentration</td>
</tr>
<tr>
<td>LD&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Median Lethal Dose</td>
</tr>
<tr>
<td>LF</td>
<td>Lethal Factor</td>
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<tr>
<td>LRRI</td>
<td>Lovelace Respiratory Research Institute</td>
</tr>
<tr>
<td>LVS</td>
<td>Live Vaccine Strain</td>
</tr>
<tr>
<td>MINA</td>
<td>Monoisonitrosoacetone</td>
</tr>
<tr>
<td>MIPLD</td>
<td>Mouse Intraperitoneal Lethal Dose</td>
</tr>
<tr>
<td>MIPLD&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Median Mouse Intraperitoneal Lethal Dose</td>
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<tr>
<td>MMWR</td>
<td>Morbidity and Mortality Weekly Report</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
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<tr>
<td>MRV</td>
<td>Military Research Volunteer</td>
</tr>
<tr>
<td>MVA</td>
<td>Modified Vaccinia Ankara</td>
</tr>
<tr>
<td>MyD88</td>
<td>Myeloid Differentiation Protein 88</td>
</tr>
<tr>
<td>NATO</td>
<td>North Atlantic Treaty Organization</td>
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<tr>
<td>OPH</td>
<td>Organophosphorus Hydrolase</td>
</tr>
<tr>
<td>OTSG</td>
<td>U.S. Army Office of the Surgeon General</td>
</tr>
<tr>
<td>PA</td>
<td>Protective Antigen</td>
</tr>
<tr>
<td>PARP</td>
<td>Poly ADP Ribose Polymerase</td>
</tr>
<tr>
<td>PGA</td>
<td>Poly-Gamma-D-Glutamic Acid</td>
</tr>
<tr>
<td>PIV</td>
<td>Pseudoinfectious Virus</td>
</tr>
<tr>
<td>QFS</td>
<td>Q Fever Fatigue Syndrome</td>
</tr>
<tr>
<td>rPA</td>
<td>Recombinant Protective Antigen</td>
</tr>
<tr>
<td>SEARCH</td>
<td>System for Evaluation and Archiving of Radiation Accidents Based on Case Histories</td>
</tr>
<tr>
<td>SEB</td>
<td>Staphylococcal Enterotoxin B</td>
</tr>
<tr>
<td>SNS</td>
<td>Strategic National Stockpile</td>
</tr>
<tr>
<td>UK</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>U.S.</td>
<td>United States</td>
</tr>
<tr>
<td>USAMRIID</td>
<td>United States Army Medical Research Institute of Infectious Diseases</td>
</tr>
<tr>
<td>VEE</td>
<td>Venezuelan Equine Encephalitis</td>
</tr>
<tr>
<td>VIG</td>
<td>Vaccinia Immune Globulin</td>
</tr>
<tr>
<td>VX</td>
<td>Methylphosphonothioic Acid Nerve Agent</td>
</tr>
<tr>
<td>WEE</td>
<td>Western Equine Encephalitis</td>
</tr>
<tr>
<td>WWII</td>
<td>World War Two</td>
</tr>
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</table>
This is the fourth in a series of annual reviews on the extension of the AMedP-8(C) methodology to New Agents, Materials, and Conditions. This annual review focuses primarily on newly available data that can be used to update existing agents or effects in the methodology. A literature review was conducted to 1) identify data sources immediately useful for updating AMedP-8(C) human response parameters or otherwise modifying the methodology and 2) help inform future analyses and serve as a starting point for related research efforts. Topics for future analysis were identified, and the level of effort to carry out each analysis was estimated. Recommended future efforts fell into three broad groups: editorial changes to the text of future versions of AMedP-8 or related documents, the incorporation of new data into existing AMedP-8(C) models, and the comparison of AMedP-8(C) models to other published models for validation or revision.