Animal Models for Assessing Countermeasures to Bioterrorism Agents

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ANIMAL MODELS FOR ASSESSING COUNTERMEASURES TO BIOTERRORISM AGENTS

Committee on Animal Models for Assessing Countermeasures to Bioterrorism Agents

Institute for Laboratory Animal Research
Division on Earth and Life Studies

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This report, *Animal Models for Assessing Countermeasures to Bioterrorism Agents*, has been reviewed in draft form by individuals chosen for their diverse perspectives and expertise in accordance with procedures approved by the Report Review Committee of the National Research Council (NRC). The purpose of this independent review is to provide candid and critical comments that will assist the Committee on Animal Models for Assessing Countermeasures to Bioterrorism Agents in making its published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberation process. The Committee thanks the following individuals for their review of the draft report:

Joy Cavagnaro, Access BIO, LC
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Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations, nor did they see the final draft of the report before its release. The review of this report was overseen by Leslie Z. Bennett, University of California-San Francisco and Ann Arvin, Stanford University School of Medicine. Appointed by the NRC, they were responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

The Committee expresses its appreciation to Karen Goldenthal who served on this panel until July 2011. The Committee also extends its appreciation to Judith Hewitt for the white paper *Developing Animal Models for Use in Animal Rule Licensure: The NIAID Approach*. Finally, the Committee thanks the speakers who addressed the Committee in the public meetings of September and November 2009, and February 2010.

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George W. Korch, Jr., *Co-Chair*
Steven M. Niemi, *Co-Chair*
Committee on *Animal Models for Assessing Countermeasures to Bioterrorism Agents*

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Summary

The Transformational Medical Technologies (TMT[1]) has been a unique component of the U.S. Department of Defense (DoD) medical biodefense efforts since 2006. Its mission is to advance countermeasure research and development in support of the broader goal of the DoD to protect warfighters from emerging infectious diseases and future genetically engineered biological weapons. The TMT, using advanced science and technology approaches, focused on the development of broad-spectrum countermeasures that target common host and pathogen pathways or enhance the host's immune response. Many of these pathogens are lethal or cause such debilitating diseases in humans that it is ethically inappropriate to test the efficacy of these countermeasures in human volunteers.

In lieu of human participants, these products may be tested in animals and approved for human use under the provisions of the Food and Drug Administration (FDA)'s 2002 Animal Rule.[2] The reliance on animal models for the development and licensure of medical countermeasures against biothreats is challenging for a number of reasons. In many cases, qualified animal models that can predict efficacy of new drugs or biologics are not available. There are numerous challenges in establishing new models to replace or complement existing ones in order for "certain new drugs and biological products that are intended to reduce or prevent serious or life-threatening conditions can be approved for marketing based on evidence of effectiveness derived from appropriate studies in animals, without adequate and

[1]The Transformational Medical Technologies Initiative (TMTI) is referred to as Transformational Medical Technologies (TMT) throughout the report. In 2011 the Department of Defense moved the TMT to a Program Manager under the auspices of the Joint Program Executive Office for Chemical and Biological Defense, as the efforts have matured to advanced development. The Committee has addressed its report to the TMT.

[2] The Animal Rule "provides for approval of certain new drug and biological products based on animal data when adequate and well-controlled efficacy studies in humans cannot be ethically conducted because the studies would involve administering a potentially lethal or permanently disabling toxic substance or organism to healthy human volunteers and field trials are not feasible prior to approval. Under this rule, in these situations, certain new drug and biological products that are intended to reduce or prevent serious or life-threatening conditions can be approved for marketing based on evidence of effectiveness derived from appropriate studies in animals, without adequate and well-controlled efficacy studies in humans (§ 314.126)" (21 CFR Parts 314 and 601 [2002]).

well-controlled studies in humans..." (FDA 2002, p 37989). There are also challenges in establishing sustainable and appropriate alternatives to the use of animal models for the development of countermeasures against biothreats.

CHARGE TO THE AUTHORING COMMITTEE

The DoD asked the National Research Council (NRC) to prepare a consensus report that would address the challenges stemming from developing and testing medical countermeasures against biothreat agents in animal models. The ad hoc Committee on Animal Models for Assessing Countermeasures to Bioterrorism Agents was charged with responding to three tasks:

1. Evaluate how well the existing TMT-employed or candidate animal models reflect the pathophysiology, clinical picture, and treatment of human disease as related to the agents of interest.
2. Address the process and/or feasibility of developing new animal models for critical biodefense research, placing emphasis on the need for a robust and expeditious validation process in terms of FDA's Animal Rule.
3. Evaluate alternatives to the use of animal models based on the premise of the Three Rs (i.e., refinement, reduction, and replacement of animal use; such venues would include but not be limited to in vitro work, computational modeling, new biotechnological tools, surrogate diseases, etc.) vis-à-vis the Animal Rule and FDA licensure. The evaluation will also consider the development of more humane models for infectious diseases research that do not incorporate death as an endpoint (i.e., humane endpoints).

The Committee approached its task by considering scientific, legal, ethical, and veterinary medicine-related elements to formulate its response to these three tasks. The Committee held two public meetings with invited experts: scientists, laboratory animal veterinarians, public health experts, policymakers, and representatives of the military (see Appendix D). It also solicited a white paper on the approach and effort to develop animal models for licensure under the Animal Rule of the National Institute for Allergy and Infectious Diseases (see Appendix C). The report was organized following the three elements of the Statement of Task with an additional chapter on ethical and regulatory challenges encountered in developing countermeasures. The Committee identified scientific and technical issues that affect the value and relevance of animal models to "provide substantial evidence of the effectiveness of these products" (FDA 2002, p 37989) under the conditions imposed by the Animal Rule and provided conclusions accordingly. The Committee did not consider animal models used to evaluate safety of products approved under the Animal Rule. The Committee did not evaluate the Animal Rule or the FDA's approach to assess product efficacy under the rule.

This report makes two principal points:

1. A comprehensive strategy to improve data gathering and data sharing from animal models (or their alternatives) would significantly increase the efficiency and productivity of research into bioterrorism countermeasures if it includes:
    - compartmentalization;[3]
    - the use of systems biology and in vitro/in silico methods;

[3] Experiments that yield information from components of the animal (organs, cells, and systems) rather than data derived from the whole organism (for additional discussion see Chapter 4).

- systematic collection of and access to experimental data;
- publication of negative results;
- enhanced collection and analysis of human data; and
- added clinical veterinary care.

2. This strategy would improve the humane use of laboratory animals in accordance with the principles of the Three Rs (i.e., refinement, reduction, and replacement of the use of animals in research).

The Committee's conclusions and recommendations follow.

CONCLUSIONS AND RECOMMENDATIONS

Evaluation of Current and Future TMT-Used Animal Models (Chapter 2)

Currently available animal models for the development of countermeasures against biothreats are imperfect representations of the human-pathogen interaction, especially with regard to their substitution for "adequate and well-controlled efficacy studies in humans" (FDA 2002, p 37989). Their limitations include:

- lack of sufficient human data and knowledge of the natural history of the diseases or threats of interest;
- methodological differences due to interspecies and intraspecies variability and the constraints imposed by working in biocontainment facilities; and
- for some conventional diseases, animal models have been shown to be unreliable surrogates for, or predictors of, efficacy and safety, as indicated by experience with product development and clinical trials.

However, the Committee concludes that the animal models available at the present time remain central for understanding pathogenesis and correlates of protection to inform effectiveness of therapeutics or vaccines developed under the Animal Rule. Because these models are complex and expensive to develop, depend on the use of large numbers of animals, and are restricted by work in biocontainment facilities, the Committee believes that the purpose of current models needs to be reevaluated—focusing on a broader application profile, i.e., product-neutral, so that more than one countermeasure may be developed, potentially including countermeasures to "unknown-unknowns." In doing so, the limitations outlined above need to be taken into consideration, i.e., (1) that methodological differences may account for common failings of animal models to correctly represent the human condition; and (2) that the collection of human data is of utmost importance in order to verify the usefulness and augment the strengths of available models.

Developing New Animal Models for Biodefense Research (Chapter 4)

Development of new animal models for biodefense research cannot resolve the limitations of the currently available ones (i.e., paucity of human data, significant costs, and methodological differences). **Therefore the Committee concludes that focusing on the creation of new animal models is not warranted at this time**. It would be more useful to the TMT to support the qualification (vs. validation) of currently available animal models, as it would advance the predictive capacity of animal-derived

data for the human response. **The Committee recommends that the TMT adopt the following strategies:**

- **To address the dearth of data from human populations, expand the collection of data from patients in outbreak zones and from postmarketing studies. In addition, expand the acquisition of data from phase 1 safety trials by (1) increasing the duration of these trials; (2) diversifying the enrolled subjects to mirror the general population; and (3) including the anticipated treatment in the field as part of the trial protocol.**
- **To control interspecies variability and improve the comparativeness of infectious disease models across different species, adopt the concept of compartmentalization. As each species is made up of a variety of physiological compartments that contribute to the host response to an infectious agent, compartmentalization is a strategy to compare the systems and pathways that lead up to the host response within a species, across species, and with humans rather than focusing on a single gene or protein or particular genes or proteins.**
- **To support the qualification of animal models as an alternative to validation, establish the compartmentalized model's scientific relevance and reproducibility across different methods and laboratories. These comparative datasets may subsequently be used to define appropriate criteria to characterize or qualify vs. validate the animal model.**

Alternative Approaches to Animal Testing for Biodefense Countermeasures (Chapter 5)

In 1959, Russell and Burch published a practical approach to refine, reduce, and replace the use of animals in research, known as the Three Rs. The Three Rs are applied to (1) refine the experimental and husbandry methods to enhance animal well-being and minimize or eliminate pain and distress; (2) reduce the number of animals needed in experimentation; and (3) replace (in absolute or relative terms) the use of animals. However, the premise of the Animal Rule is that the effectiveness of new drugs and biologics when human efficacy studies are not ethical or feasible may be demonstrated in appropriate studies in animals. Currently, the development of countermeasures to biothreats depends on animal models for efficacy testing of these products in lieu of human clinical trials. **The Committee concludes that absolute replacement of animal models in countermeasure development is not possible at this time and that in vitro and in silico methods are not advanced enough yet (in part due to absence of human data) to reliably replace animals in biodefense research.**

The Committee recommends that the TMT undertake an analysis of the discovery, development, and approval process for medical countermeasures to identify (1) where the most important scientific gaps exist in terms of utilizing alternative methods to animal models and how to address them; (2) the specific areas where the use of in vitro and in silico methods could be sufficient or as an adjunct to the use of animals; and (3) the criteria for choosing and utilizing the most suitable technologies to replace animal use in biodefense research.

Original data and information from animals and humans should be collected systematically, consistently, and accurately and be made available to the research community. Sharing of both positive and negative data will enable progress toward standardization of methods and qualification of models, and may also help in the event of an "unknown-unknown" emergency. It will also address ethical concerns regarding the potential nonproductive or duplicative use of animals or the unnecessary duplication of studies and waste of resources.

The Committee concludes that changing the standard practice of animal experimentation to approximate the clinical course of treatment that humans may receive could provide a more reasonable expectation of the usefulness of certain countermeasures during development. Consequently, the provision of supportive care is a means to improve data gathering from animal

models. Details of supportive care should be discussed with the FDA early in the planning stages before studies are initiated. As a reasonable measure to incorporate in the study design, it is not only a more humane approach but may allow fewer animals to be used in accordance with the Three Rs. Experience from such experimental protocols may be helpful in the event of countermeasure trials against an "unknown-unknown." The Committee recognizes that the nature of biocontainment imposes difficulties in the implementation of the above. **Therefore, the Committee recommends that the TMT define the basic principles of such an approach, including guidelines for the care and use of animals in research in biocontainment facilities.**

Finally, the Committee concludes that the potential advances in knowledge and benefits to the warfighters should be weighed against the duration and severity of animal pain and distress. Further, the Committee believes that the application of refinement strategies and reduction approaches (as discussed in Chapter 5) could improve laboratory animal welfare and safeguard the quality of biodefense research. Moreover, the recommended comprehensive strategy of implementing the Three Rs, incorporating compartmentalization, and enhancing collection and analysis of human data reduces the dependency of this field of research on nonhuman primates by maximizing the value of data derived from all research. **The Committee recommends that, where possible, the TMT should encourage efforts to replace nonhuman primates as the animal of choice in biodefense research.** Such efforts coupled with unhindered access to data and publishing of all results—including negative ones—are critical steps to ensure that this data are beneficial, animals are used judiciously, and unnecessary duplication of work is avoided.

1

Introduction

THE NATURE OF THREAT

Infectious diseases have always been with us, and always will. As Nobel Laureate Dr. Joshua Lederberg observed (Lederberg 2000), it is a competition between their genes and our brains. In addition to advances in medical products (such as drugs and vaccines) to treat or prevent natural infectious agents, multiple voices have argued that current advances in biological research and biotechnology would enable the development of bioengineered pathogens (Lindler et al. 2005; Petro et al. 2003), called by the U.S. Department of Defense (DoD) the "unknown-unknowns"[1] because of their unknown profile and their unknown potential threat to warfighters and the public at large.

The United States and other governments have identified both the need to prevent the development of such designed pathogens and the need for a strategy to develop medical interventions, that is, medical countermeasures, against this unfamiliar group of potential infectious agents.

Preventing the development of biothreats would rely on predictive reasoning or covert discovery of the effort to develop such agents. However, while access to the methodologies, materials, and knowledge base of molecular biology particularly and bioscience more generally increases, the size and scale of such intelligence- and data-gathering capability decrease, making the reliable detection of such efforts possibly more difficult.

A responding strategy for addressing the threat of these novel unknown-unknowns is to thoroughly study the foundations and patterns of host-parasite or host-microbe evolutionary dynamics and patterns of interaction. All human infectious diseases had an origin in some preceding host-parasite system either of ancient or more recently recognized origin. The evolution of variola virus (causative agent of smallpox) is traced to an East African rodent host species 16,000-68,000 years ago, and *Yersinia pestis* is thought to have diverged from a *Y. pseudotuberculosis* lineage over the past 1,500-20,000 years, possibly as the bacterium adapted to life in the flea host (Achtman et al. 1999; Eppinger et al. 2010). An example of a modern emerging human disease is HIV whose origin is in nonhuman

[1] The term "unknown-unknown(s)" refers to pathogen(s) that may not be known or knowable because they currently may not exist. Due to the current or future possibility that they may exist, they are considered potential threats (e.g., a novel, genetically engineered, or created pathogen).

primates (Rambaut et al. 2004). An infinite array of patterns that yield favorable opportunities for a pathogen probably does not exist, and conversely, defense mechanisms that the host is capable of generating are probably few.

If the host is a collection of different environments and opportunities for exploitation by a pathogen, then a successful pathogen must bring the proper tools to exploit that opportunity (tailored adaptation strategy), and those tools probably fit into (recognizable) major patterns (mechanisms of pathogenesis). Any pattern or patterns of specific adaptations by these pathogens may be targeted for medical countermeasures, and mechanisms of pathogenesis that are similar or shared by different host species (human and nonhuman) may be used to demonstrate comparable efficacy of countermeasures when such assessment cannot be performed in humans.

ADDRESSING THE UNKNOWN THREAT THROUGH THE TRANSFORMATIONAL MEDICAL TECHNOLOGIES INITIATIVE[2]

The Transformational Medical Technologies (TMT; see Box 1-1) reflects a key transition point in the DoD's philosophy about biological threats and the approach to developing medical countermeasures (MCMs). The overarching strategy for the TMT, conceived for the Quadrennial Defense Review (QDR) in 2006 by the DoD in its *Chemical and Biological Defense Program Medical Research and Development, Testing & Evaluation (RDT&E) Plan*, is as follows:

- The key to defending against unpredictable or unknown threats (e.g., bioengineered pathogens) lies not in expending resources to uncover the types of advanced agents that humans could face but rather in exploring and comparing the underlying pathophysiological patterns in the interaction of pathogen and host by using advanced scientific approaches, such as systems biology.
- In addition to the traditional method of looking for vulnerable pathogen targets, the strategy assumed the possibility of targeting broadly used host pathways for intervention. The TMT could ostensibly achieve broad protection against a variety of threats by looking at both host- and pathogen-based targets.
- The TMT's strategy hypothesized that the key to defending against unknowns could be found in understanding potentially commonly evolved pathways and developing medical countermeasures focused on *pathogenesis patterns*, rather than on specific pathogens and the traditional "one-bug, one-drug" approach.
- The strategy suggested that pathogens that occupy similar "pathogenesis niches", e.g., viruses that produce hemorrhagic responses in hosts, or bacteria that survive by exploiting an intracellular niche, acquired evolutionarily similar mechanisms or biochemical tools to achieve these niche-specific outcomes.

[2] The Transformational Medical Technologies Initiative (TMTI) became Transformational Medical Technologies (TMT) and is referred to as such throughout the report. In 2011 the Department of Defense moved the TMT to a Program Manager under the auspices of the Joint Program Executive Office for Chemical and Biological Defense, as the efforts have matured to advanced development. The Committee has addressed its report to the TMT.

BOX 1-1
Transformational Medical Technologies Initiative

The Department of Defense's Transformational Medical Technologies Initiative (TMTI; now known as Transformational Medical Technologies; TMT) was organized in 2006 to boost countermeasure development with a "transformational" approach focusing on countermeasures with a broad enough therapeutic or preventive profile to defend against unanticipated and novel threats. The methods used to develop these countermeasures should be quickly adaptable to new threats once the genomic sequence of the pathogens is determined. The TMTI was to be transformational in shepherding the development of medical countermeasures and diagnostic products from early research through development phases - an "end-to-end" approach.

The development of medical countermeasures and diagnostic products by the TMTI was notable. The countermeasure work that is most relevant to this study includes investment in a wide range of projects including so-called platforms - technologies that can be used to quickly produce countermeasures against different targets. One example of the platform approach is the synthesis of antisense oligonucleotides that target the sequence of a biothreat agent by attacking the agent RNA with a complementary, or "antisense", synthesized oligonucleotide strand that binds the agent's RNA and leads to its destruction by the host cell (see Warren et al. 2010). Other funded research included efforts to create potentiators of the immune system.

In 2010, the TMTI became a program, instead of an initiative, known simply as Transformational Medical Technologies, or TMT. In 2011 the Department of Defense moved the TMT to a Program Manager under the auspices of the Joint Program Executive Office for Chemical and Biological Defense, as the efforts have matured to advanced development.

The QDR directed the DoD "to develop broad-spectrum MCMs against the threat of genetically engineered bio-terror agents" (DoD 2006, p 5), recognizing that emerging disease and potentially genetically engineered pathogens, could be leveraged as effective agents to wage "asymmetric warfare." As a result, the TMT was created to provide new solutions for the warfighter that could be broadly generalized, even to unknown threats. The TMT was designed to incorporate systems biology approaches to understand patterns of pathogenesis for the purposes of developing and targeting medical countermeasures at various single or combined targets to achieve a broad level of protection or treatment (see Figure 1-1 for notional approach).

The program's principal thrust has been to build a capability to respond to an event by using platform technologies to identify and counter unknown biological threat agents. Technologies have been developed that accelerate the process of definitive pathogen characterization, as well as the design and deployment of MCMs. The response capability that was tested in a real-world situation against the 2009 H1N1 influenza outbreak resulted in an effective medical countermeasure against the H1N1 virus. The countermeasure was produced using an antisense oligonucleotide therapeutic platform.

Another core area of the TMT has been the development, through Federal Drug Administration licensure, of "broad-spectrum" therapeutics. A defining feature of this MCM effort has been the use of intervention strategies that target multiple classes of pathogens, as opposed to the conventional "one-bug, one-drug" paradigm. Such approaches may defeat pathogens directly (through antibiotics or antivirals), exploit host targets attacked by multiple threat agents, or enhance host defenses by modulating the host's immune response. An example of this approach is the targeting of the human protein TSG101, the product of the tumor susceptibility gene 101 that participates in the intracellular movement of proteins. This protein is "hijacked" by viral components to bring intracellular proteins to the surface of the cell for eventual virus assembly and budding (e.g., HIV and Ebola; Martin-Serrano et al. 2001). Once TSG101 is exposed on the cell surface it can be directly targeted using monoclonal antibodies to then help eliminate infected cells. Developed with TMT funding, one monoclonal antibody has demonstrated in vitro efficacy against many virus types, including HIV-1 and drug-

resistant HIV (Chen et al. 2010), and all forms of influenza (both seasonal and pandemic; Bonavia et al. 2010). Recent work in murine models has shown protection against a number of filoviruses through the development of broad-spectrum antivirals (FGI-106, FGI-103) whose cellular target remains undefined (Aman et al. 2009; Warren et al. 2010).

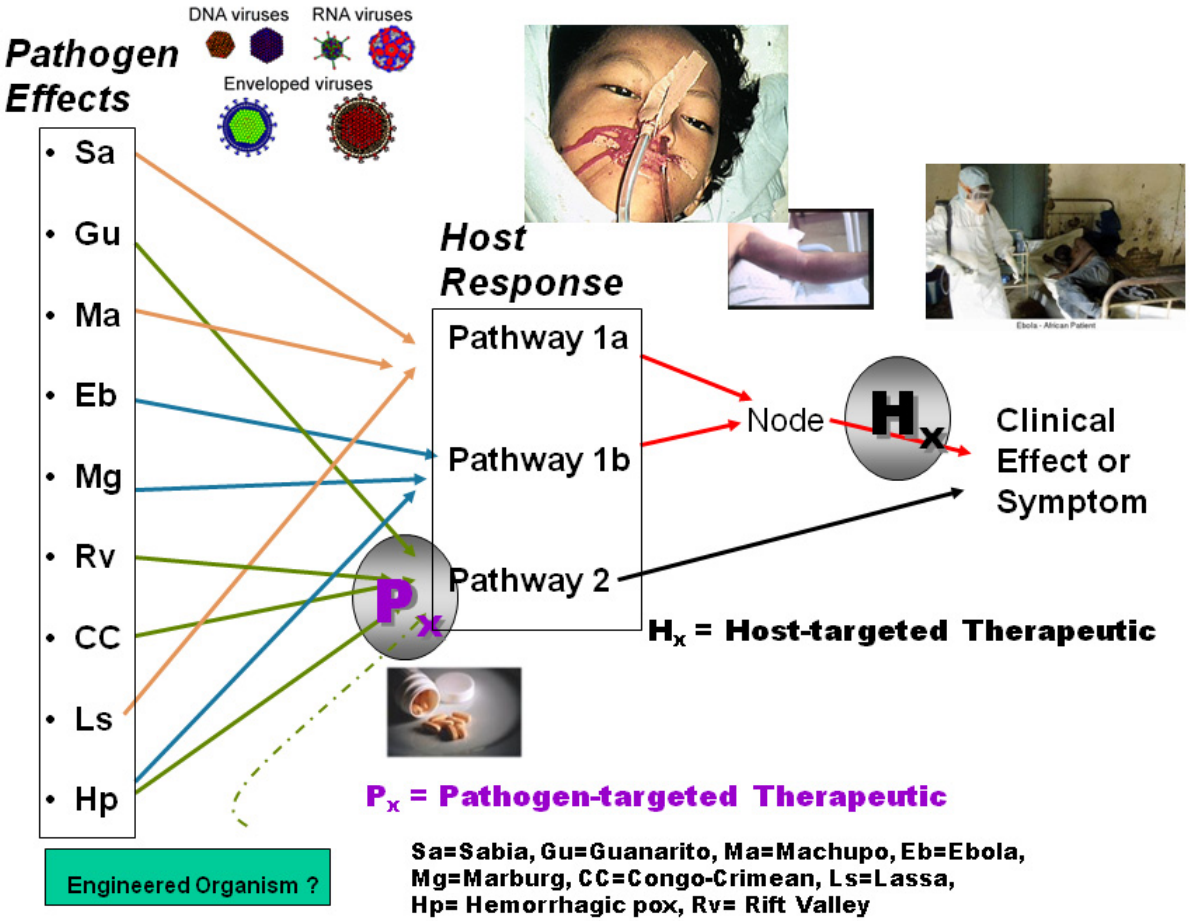

FIGURE 1-1 TMT concepts for broad capability against general categories or clusters of pathogens. These concepts exploit a particular life-history strategy, thereby achieving protection against unknown pathogens with similar pathogenesis patterns. In this graphic representation, major hemorrhagic fever viruses from different taxa (on the left side represented by two letters) are hypothesized to use one of three pathogenesis pathways to successfully infect a host, while the host has several major specific response pathways. Broad ability to defend against this entire class of pathogens through treatment would depend on developing a combination of therapeutics that act at several different but complementary points within the overall pattern (designated by P_x and H_x), so that any unknown or engineered organism attempting to exploit this potential pathogenesis model would effectively be prevented from fulfilling its goal.

According to the DoD, the TMT is unique among U.S. government medical countermeasure efforts because it supports the full spectrum of drug development by funding basic research through advanced product development. Investigational New Drug (IND) filings for two hemorrhagic fever viruses (Marburg and Ebola) have resulted from this program, and additional IND filings are anticipated in the near future. The TMT's integrated product development program has also sought out

and created public-private partnerships with pharmaceutical companies whose longer-term goals are compatible with the infectious-disease interests of the program. The program has become an integral component of the larger national effort to combat biological threats, whether they result from acts of terrorism or emerging infectious disease.

STATEMENT OF TASK

The study presented here was identified as an important topic by the National Research Council's (NRC's) Standing Committee on Biodefense for the Department of Defense. The Standing Committee was organized by NRC in 2007 at the request of the Office of the Secretary of Defense (Special Assistant for Chemical and Biological Defense and Chemical Demilitarization Programs). The Committee's work focused on the DoD's TMT and the challenges faced by the broader community in the development of medical countermeasures against biothreat agents. The TMT's goals and approach were to be transformational (see Box 1-1). In focusing on bottlenecks and obstacles to the development of medical countermeasures the Standing Committee became aware of the Food and Drug Administration's (FDA's) Animal Rule (see Appendix A; 21 CFR Parts 314 and 601 [2002]). The Animal Rule is largely considered a step forward in addressing the fact that the efficacy of countermeasures against most biothreat agents cannot be tested in humans because it is unethical for humans to be given the disease against which the countermeasures are intended to work. However, while the FDA's action to create a pathway for testing the effectiveness of countermeasures without human clinical trials was well received, experience since its promulgation demonstrated that it is not a facile pathway for assessing the efficacy of a countermeasure in humans based on the product's efficacy in animals. This past decade has shown that the Animal Rule presents its own set of challenges, including developing appropriate animal models of pathogenesis and extrapolating results from animals to humans. Recognizing the need for focused attention on the issues, the Standing Committee and the DoD asked the NRC to organize a separate ad hoc committee to produce a report addressing issues related to animal models for testing countermeasure efficacy (see the complete Statement of Task in Appendix E).

Although this report was funded with the DoD and TMT's needs in mind, the charge was to address a challenge that is widely accepted to be a major obstacle for the entire scientific and research community working on the development of medical countermeasures. The Committee on Animal Models for Assessing Countermeasures to Bioterrorism Agents was asked to:

1. Evaluate how well the existing TMT-employed or candidate animal models reflect the pathophysiology, clinical picture, and treatment of human disease as related to the agents of interest.
2. Address the process and/or feasibility of developing new animal models for critical biodefense research, placing emphasis on the need for a robust and expeditious validation process in terms of FDA's Animal Rule.
3. Evaluate alternatives to the use of animal models based on the premise of the Three Rs (refinement, reduction, and replacement of animal use; such venues would include but not be limited to in vitro work, computational modeling, new biotechnological tools, surrogate diseases, etc.) vis-à-vis the Animal Rule and FDA licensure. The evaluation will also consider the development of more humane models for infectious diseases research that do not incorporate death as an endpoint (i.e., humane endpoints).

APPROACH BY THE COMMITTEE

In the chapters to follow, the Committee on Animal Models for Assessing Countermeasures to Bioterrorism Agents lays out its findings and offers potential solutions for the DoD to address the challenge of exclusively using animal models to demonstrate the effectiveness of a medical countermeasure in lieu of human efficacy data. The Committee did not consider animal models to evaluate the safety of products developed under the Animal Rule, as under the rule's provisions "safety evaluation of products is not addressed in this rule" (FDA 2002, p 37989). Further, the Committee did not evaluate the Animal Rule or the FDA's approach to assess product efficacy under the rule.

Chapter 2 looks at the adequacy of current animal model systems including an assessment of the data provided by these models versus available human data for filovirus-induced hemorrhagic fevers, anthrax, and tularemia. Chapter 3 discusses the history of the Animal Rule and relevant ethical issues. Chapter 4 explores the need for additional animal models to augment current capabilities and introduces the issue of qualification of models to be used for both hypothesis testing and regulatory purposes (i.e., toxicology studies). It suggests the compartmentalization of an animal model to match specific aspects of efficacy demonstration to individual components of the model rather than to results from the whole organism. Finally, chapter 5 considers what approaches and refinements should be applied now to current animal models for the TMT and recommends the exploration of advanced technologies and new types of genetically modified animals. It further discusses the potential value of supplementing the veterinary and clinical care of an experimental animal subjected to these pathogens to align with the clinical treatment received by the human patient.

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2

Evaluation of Current and Future TMT-Used Animal Models

This chapter examines how well specific animal models against biothreats of interest to the Transformational Medical Technologies (TMT) reflect various aspects of the human diseases for which medical countermeasures are being developed. As explained in the Introduction, the TMT seeks to identify and develop new or repurposed medical countermeasures that may have broad-spectrum capability, that is, target a number of pathogens with similar mechanisms of disease causation and pathogenesis. This approach is focused on two major groups, hemorrhagic fever viruses and intracellular bacterial pathogens. The Committee on Animal Models for Assessing Countermeasures to Bioterrorism Agents thinks that currently available animal models for these biothreats, while necessary, are imperfect representations of every aspect of human-pathogen interaction especially with regards to their substitution for "adequate and well-controlled efficacy studies in humans" (FDA 2002, p 37989). Given the ethical mandate of the Animal Rule to not harm human participants in clinical trials that "would involve administering a potentially lethal or permanently disabling toxic substance or organism" (ibid.), these models most likely represent the best approach to develop and test countermeasures and the current efforts have performed as well as could be expected given the limitations listed below. These limitations are critical components to be considered when evaluating the utility of an animal model for efficacy studies[1] of the known or unknown pathogens of interest to the TMT:

- Lack of sufficient human clinical data (that is, reliable and sophisticated human clinical markers) and knowledge of the natural history[2] of these diseases or threats of interest may hinder the successful correlation of the animal models to the infectious diseases of interest. The more scant the human data, the greater the uncertainty of relevance of the animal model.

[1] The Committee did not consider animal models used for safety evaluation of products developed under the Animal Rule, as "safety evaluation of products is not addressed in this rule" (FDA 2002, p 37989).
[2] Natural history refers to the progression of a disease without any intervention.

- Both interspecies and intraspecies variability and the constraints imposed by working in biocontainment facilities lead to methodological differences and results that may not be translatable or comparable across different animal models of the same disease. This is particularly relevant to the anticipated clinical experience of human patients.[3]
- Experience with product development and clinical trials for some conventional diseases indicate that animal models often are unreliable surrogates for, or predictors of, efficacy and safety.[4,5]

Historically, animal models have been relied upon to provide preliminary efficacy data for therapeutics against infectious diseases in support and justification of subsequent definitive efficacy studies in human participants to obtain regulatory approval by the Food and Drug Administration (FDA). Because the preclinical data would be evaluated in the context of knowledge from human studies, any deficiencies in data correlation and extrapolation from the animal models to the human condition would presumably be compensated for by the actual data collected during the human studies. Biothreats represent a special problem in that efficacy studies before an actual event are unlikely to take place. In addition, the actual risk of a biothreat attack is difficult to ascertain. These difficulties are even more pronounced in the case of the "unknown-unknowns".[6]

Comparing the evaluation process for bioterrorism countermeasures following the preclinical development stage with that for drugs for which human efficacy studies are possible puts in better perspective the regulatory challenges with which the countermeasure development for TMT (or other biodefense) products is beset. Under optimal circumstances, the current process from drug discovery to FDA approval takes an average of 10 to 15 years and costs more than $1 billion (Tamimi and Ellis 2009). According to some estimates the developmental cost of a single drug has soared from $1.1 billion in 1995 to $1.7 billion in 2002, factoring in the costs of failed prospective drugs (Crawford 2004; Mundae and Östör 2010). Those figures apply equally to biopharmaceuticals and small molecules (DiMasi and Grabowski 2007). To date only about 8% of drugs that successfully enter phase 1 studies eventually are granted market approval by the FDA as compared with 14% in the 1980s. The success rate of pharmaceuticals from the first phase 1 study in humans to market is less than 10% (DiMasi et al. 2010).

The main causes of failure in the clinical trial setting are safety problems, which account for about 20% of the attrition rate, and lack of effectiveness, which accounts for about 40% (Kola and Landis 2004; Peck 2007). Inability to predict these failures before human testing or early in clinical trials dramatically escalates costs. In the infectious disease arena, data from the 10 largest pharmaceutical corporations in the period of 1991-2000 showed a success rate of about 15%, while the average success rate for all indications was 11% (Gilbert et al. 2003). Similarly, DiMasi and colleagues (2010) showed a success rate for systemic infectious disease of 15.6% during 1994 and 2003. It is useful to note that from 1981 to 1992 the success rate of anti-infective drugs was 28.1% and that large biopharmaceutical companies appeared to have a higher success rate of 30.2% for all indications (DiMasi 2001). A key

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[3] Lack of data sharing further compounds differences in methods or lack of reproducibility of results across models (see chapter 5 for further discussion).

[4] The limitations of animal models for other disease indications (in addition to those encountered in emerging infectious diseases or biothreats research) have been documented in a number of meta-analyses (see Macleod 2011; Perel et al. 2007; Suntharalingam et al. 2006; van der Worp et al. 2010).

[5] As discussed in *Developing Animal Models for Use in Animal Rule Licensure: The NIAID Approach* (Appendix C, p 111-112), developing animal models in biocontainment requires substantial financial and infrastructure investment.

[6] As defined in the introduction, the term "unknown-unknown(s)" refers to pathogen(s) that may not be known or knowable because they currently may not exist. Due to the current or future possibility that they may exist, they are considered potential threats (e.g., a novel, genetically engineered, or created pathogen).

question is whether medical countermeasures against emerging infectious diseases and other biothreats have a higher likelihood of success in a (theoretical) human trial. Several facts argue against this possibility and support the notion that achieving a success rate close to that of noncountermeasure drug development can only be considered a best-case scenario:

- The pathogenesis of these rare or even unknown infections is mostly unknown and cannot, therefore, guide the development process.
- The causative pathogens could be optimized to withstand interventions (e.g., via introduced antibiotic resistance).
- The clinical setting is probably one of mass infection (which may even be caused by more than one infectious agent) and thus is not comparable to randomized clinical trials of hospitalized patients.
- Most product development occurs with less than average financial support by entities not experienced in full clinical drug development.
- The restrictions imposed by biocontainment and the strong reliance on nonhuman primates limit the number of animal studies that could be done.

ANIMAL MODELS ARE ANALOGOUS, NOT HOMOLOGOUS SYSTEMS

On a number of occasions the Animal Rule has been misread resulting in the unrealistic expectation that animal efficacy studies accurately and completely reflect the human condition. Indeed, the term "model" implies that it is not intended to completely replicate the human pathophysiology but rather to provide insight into different aspects of the host-pathogen dynamic. In fact, the Animal Rule is based on the notion that there is enough similarity in the response of animals of different species to a pathogen or a group of pathogens to permit a reasoned method to evaluate product efficacy among those different species (humans being the final target). Prior knowledge of the natural history and progression of the human infection shows that the interplay between host and pathogen may or may not mimic what occurs in humans. Animal models are analogous and not homologous and, by their very nature, display a number of limitations both during different stages of the development process and in the design of the experimental protocols that are applied to these models. For the purpose of this report, homology refers to the similarity in evolutionary origin and physiological function. Analogy refers to the quality of resemblance or similarity in function or appearance but not to the similarity in origin or development (Anderson and Tucker 2006).

Although animal models incorporate a variable degree of homology and analogy, the only homologous model for a human is a human (and even among humans genetic differences affect responses and safety for vaccines and therapeutics; He et al. 2011). Most regular drugs and vaccines are tested for both safety and efficacy in clinical trials where the conditions or diseases of concern are endemic in a population, providing the opportunity to use a truly homologous model. Although efficacy data from animals have been used for decades to drive the exploration of new countermeasures to biological agents and toxins, only in the last decade has there been a need to use research data collected exclusively from analogous models (animals belonging to nonhominid taxa) for the same regulatory approval process as data from humans.

CONSIDERATIONS FOR ANIMAL MODELS FOR COUNTERMEASURE DEVELOPMENT

Two of the conditions of the Animal Rule that have to be met for the FDA to use evidence of efficacy derived from animal studies are the following:

1. There is a reasonably well-understood pathophysiological mechanism of the pathogenicity of the infectious agent and its prevention or reduction of symptoms by the product.
2. The effect is demonstrated in more than one animal model (animals belonging to at least two different species) expected to react with a response predictive for humans unless the effect is demonstrated in animals belonging to a single animal species that represent a sufficiently well-characterized animal model for predicting the response in humans (FDA 2002).

These two conditions often provide some of the biggest hurdles in developing an animal model for countermeasure development. For example, the first condition infers that a large amount of human clinical and pathophysiological data is available to compare with the data derived from the animal model. In many cases, there are sparse to no data on some of the biothreat infections because of their rare geographic distribution and infrequent rate of occurrence. Although autopsy data may be available, they provide little information about the natural history of disease and may be influenced during the terminal stages of infection by a variety of epiphenomena, such as the lack of supportive treatment or the presence of secondary systemic failure. Pathogens with tropism for animals of a single species make the fulfillment of the second condition particularly difficult. Variola virus, the causative agent of smallpox, is a prime example of this problem because in nature it infects only humans. Developing working animal models for variola to replicate the natural progression of smallpox is very difficult if not impossible. Furthermore, although in some cases the model may reflect different aspects of the pathophysiology of smallpox, the actual progression of the illness in animals may be quite different from that observed in humans. The rabbit model for pulmonary anthrax is an example of the latter; the difference in progression can create significant problems for protocols related to product development (see further discussion on page 31).

The significance of the majority of pathogens currently viewed as priorities for biodefense research changed over the last ten years in response to the September 11, 2001, events. Despite the changed status, funds for research of these pathogens were minimal, numbers of researchers specializing in this field were low, and overall research progress was slow. Impeding progress even further, a considerable number of these agents are categorized as Risk Group 3 and 4 pathogens for biosafety and security reasons (Select Agents Regulations; 7 CFR Part 331; 9 CFR Part 121; 42 CFR Part 73), therefore requiring biosafety level 3 or 4 (BSL-3 or -4) containment facilities for any research to be conducted in the United States (ibid.). Accordingly, animals can be experimentally infected with these pathogens only in the appropriate animal biosafety level containment facilities (ABSL-3 or -4).

The following review of several pathogens provides a broad representation of the current status of animal models being developed for efficacy testing and highlights specific challenges common among other models in the context of the Animal Rule, as depicted in Table 2-1.

TABLE 2-1 Current State of Animal Model Development for Selected Pathogens in the Context of the Animal Rule

	Filoviruses	Variola virus	*Francisella tularensis*	*Bacillus anthracis*
Research and product discovery	Rodent and nonhuman primate models (Falzarano et al. 2011)	Surrogate models used with other poxviruses	Predominantly murine models	Large body of data
Proof of principle	Yes in rodent models	Yes for surrogate models	Historical information from human challenges	Large body of data
	FDA Animal Rule Applied for Product Transition			
1. Well-understood pathophysiology	Limited understanding due to lack of human data	Limited understanding of humans (Stanford et al. 2007)	Strong pathology but basic mechanistic information lacking	Toxin-mediated bacteremia
2. Animals of more than one species	Mouse, guinea pig, hamster, nonhuman primates, but limited by #1	Specific human tropism of smallpox challenging	Mouse, rat, nonhuman primates	Rabbit, nonhuman primates
3. Endpoint clearly related to human benefit	Survival, but limited by #1	Survival for surrogate models	Survival	Survival and decreased morbidity
4. Information for effective human dosing	Not applicable at this time	Yes for specific antibody responses	Correlates of protection not well defined	Reasonable correlates

FILOVIRUSES

Among viruses, TMTI focuses on those that cause viral hemorrhagic fevers (VHFs), and among those primarily on VHF-causing filoviruses (marburg-, ebola-, and "cuevaviruses"; see Table 2-2 for virus names and abbreviations). All filoviruses, except Reston virus (RESTV) and Lloviu virus (LLOV), are endemic in Central Africa. RESTV is found in the Philippines and LLOV appears to be endemic in Spain. Human filovirus disease outbreaks are rare events, limited in scope, still unpredictable, and usually occur in rural and underdeveloped areas without sophisticated medical or epidemiological infrastructure. Outbreak intervention often occurs weeks or months after index cases[7] are reported to local authorities, and Western-style medical treatment is often hindered not only by nonexistent infrastructure and the lack of trained personnel but also by cultural and especially religious, spiritual constraints. Taken together, these obstacles explain the reasons for the current paucity of available human clinical data on diseases caused by filoviruses.

The lack of basic human pathophysiological information raises the disconcerting possibility that current animal systems for filovirus infections could be only crude approximations of the human

[7] First disease case in an epidemic within a population (NIH 2011).

clinical condition rather than truly analogous models. Currently available animal "models" usually rely on the identification of particular animals that, after infection, develop a disease that has some prominent clinical or pathological markers in common with those observed in infected humans rather than on the thorough characterization of host responses that can be compared directly with those of sick humans. Thus, in the case of filoviruses, the dearth of information on the human patient prevents the development of a clinically defendable animal model. Furthermore, additional collection of human clinical data may render these animals ill-suited for the evaluation of pharmaceuticals or vaccines under the premises of the Animal Rule.

TABLE 2-2 Transformational Medical Technologies Viral Pathogen Focus Group: Filoviruses[a]

New Taxonomy	Outdated Taxonomy (Eighth ICTV Report)
Order *Mononegavirales*	Order *Mononegavirales*
Family *Filoviridae*	Family *Filoviridae*
Genus *Marburgvirus*	Genus *Marburgvirus*
Species *Marburg marburgvirus*	Species *Lake Victoria marburgvirus*
Virus 1: Marburg virus (MARV)	Virus: Lake Victoria marburgvirus (MARV)
Virus 2: Ravn virus (RAVV)	
Genus *Ebolavirus*	Genus *Ebolavirus*
Species *Taï Forest ebolavirus*	Species *Côte d'Ivoire ebolavirus* [sic]
Virus: Taï Forest virus (TAFV)	Virus: Côte d'Ivoire ebolavirus [sic] (CIEBOV)
Species *Reston ebolavirus*	Species *Reston ebolavirus*
Virus: Reston virus (RESTV)	Virus: Reston ebolavirus (REBOV)
Species *Sudan ebolavirus*	Species *Sudan ebolavirus*
Virus: Sudan virus (SUDV)	Virus: Sudan ebolavirus (SEBOV)
Species *Zaire ebolavirus*	Species *Zaire ebolavirus*
Virus: Ebola virus (EBOV)	Virus: Zaire ebolavirus (ZEBOV)
Species *Bundibugyo ebolavirus*	
Virus: Bundibugyo virus (BDBV)	
Genus "Cuevavirus"	
Species "Lloviu cuevavirus"	
Virus: Lloviu virus (LLOV)	

[a] Taxa not yet approved by the International Committee on Taxonomy of Viruses (ICTV) are in quotation marks.
SOURCE: Kuhn et al. 2010.

Filovirus Infection in Humans

The description of the clinical presentation of humans infected with filoviruses is limited. There are at least eight filoviruses, and the diseases caused by them differ substantially in case numbers, case distribution, and case fatality rates. Moreover, there are few reported cases of some of the viruses. For instance, the clinical presentation of the human disease caused by Bundibugyo virus (BDBV) was reported only once (MacNeil et al. 2010). Similarly, the paucity of information on human infection with Taï Forest virus (TAFV) (only one case described thus far and the patient survived) makes it difficult to extrapolate the symptoms and clinical progression of the disease as observed in a single patient to the population at large (Formenty et al. 1999). It remains uncertain whether humans were ever infected with RESTV or LLOV, as neither has to date been isolated from humans. However, the frequent contact of humans with RESTV-infected swine in the Philippines and the possible frequent exposure of tourists to LLOV-infected bats in Spanish caves suggest that, if humans do get infected by these ebolaviruses, the infections might be without clinical consequences (Barrette et al. 2009). Clinical presentation data on Sudan virus (SUDV) infections have yet to be statistically analyzed (Okware et al. 2002; Smith et al. 1978; WHO 1978). To date, the best-characterized filovirus diseases in human patient cohorts are those caused by Marburg virus (MARV), BDBV, and Ebola virus (EBOV), as shown in Tables 2-3, 2-4, and 2-5 (see table references, pages 22-24). It remains to be seen whether these different viruses cause fundamentally different disease pathogenesis.

Symptoms of filovirus disease are unspecific, are easily confused with many other diseases, and lack a pathognomonic marker that allows for the unequivocal diagnosis of filovirus infection. Unfortunately, autopsies of fatally infected humans have only rarely been performed, partly due to cultural constraints and partly due to safety concerns. For instance, of the 1,912 fatal filovirus infections documented between 1967 and 2010, only 31 have been pathologically examined: eight people infected with MARV/ Ravn virus (RAVV) (five in 1967 and one each in 1975, 1980, and 1987; Gear et al. 1975; Gedigk et al. 1968; Geisbert and Jaax 1998; Smith et al. 1982); 21 people infected with EBOV (three in 1976 and 18 in 1995; Murphy 1978; Zaki and Goldsmith 1999); and two people infected with SUDV in 1976 (Dietrich et al. 1978; Ellis et al. 1978). The autopsies mostly addressed gross anatomy, pathology, and standard histology and did not expand into molecular markers. The collection of more detailed clinical data has been attempted multiple times in the past and failed for numerous reasons, including lack of accessibility to patients, knowledge of ongoing outbreaks, or resistance of patients to be evaluated.

Autopsies of MARV/RAVV-infected patients revealed hemorrhagic diathesis into the skin (maculopapular rash), mucous membranes, and soft tissues. The gallbladders appeared normal, spleens were slightly enlarged, and lymph nodes were swollen. Focal necroses in all organs except lungs, skeletal muscles, and bones were typical findings, but inflammatory reactions were absent with the exception of testes and ovaries. MARV/RAVV was detected in macrophages, fibroblasts, hepatocytes, Kupffer cells, adrenal cells, neuroendocrine cells of the adrenal medulla, and alpha and beta pancreatic islet cells (Gear et al. 1975; Gedigk et al. 1968; Geisbert and Jaax 1998; Kuhn 2008; Smith et al. 1982). The autopsy findings in EBOV-infected patients were similar to those described for MARV/RAVV infections (Murphy 1978; Zaki and Goldsmith 1999), whereas findings in the two autopsied SUDV-infected humans remain controversial because of concomitant parasitic (trematode and nematode) infections (Dietrich et al. 1978; Ellis et al. 1978).

Relatively thorough state-of-the-art molecular analyses of filovirus-infected patients are limited to only a few studies for EBOV- and SUDV-infected patients (Baize et al. 1999, 2002; Hutchinson and Rollin 2007; Leroy et al. 2000, 2001, 2011; Rollin et al. 2007; Sanchez et al. 2004; Wauquier et al. 2010 Attempts to identify disease progression markers have shown that EBOV disease survivors mounted an

TABLE 2-3 Symptoms of Marburg Virus-Infected Humans

Clinical Symptom	Frequency Observed in Survivors (%)	Frequency Observed in Fatal Cases (%)
Abdominal pain	59	57
Anorexia	77	72
Arthralgia or myalgia	55	55
Bleeding from puncture sites	0	7
Bleeding from the gums	23	36
Bleeding from any site	59	71
Chest pain	18	4
Conjuctival infection	14	42
Cough	9	5
Diarrhea	59	56
Difficulty breathing	36	58
Epistaxis	18	34
Fever	100	92
Headaches	73	79
Hematemesis	68	76
Hematoma	0	3
Hemoptysis	9	4
Hiccups	18	44
Lumbar pain	5	8
Malaise or fatigue	86	83
Melena	41	58
Nausea and vomiting	77	76
Petechiae	9	7
Sore throat, odynophagia, or dysphagia	43	43

SOURCE: Adapted from Bausch et al. 2006.

early robust antibody (IgG) response directed against the viral nucleoprotein (NP) and matrix protein VP40, followed by clearance of viral antigen and activation of cytotoxic T cells; in fatal cases, no antibody response was observed concomitant with massive activation of monocytes and macrophages and subsequent massive lymphocyte apoptosis. Moreover, the presence of interleukins IL-1β and IL-6 during symptomatic infections could be used as predictor for nonfatal infections, whereas release of IL-10, IL-1RA, and neopterin could be used as predictor for fatal infections (Leroy et al. 2000; Wauquier et al. 2010). In SUDV patients, the interleukin profile was different; survivors had higher concentrations of interferon α (IFN-α) and fatal cases had higher concentrations of IL-6, IL-8, IL-10, and macrophage inflammatory protein 1β (MIP-1β; Hutchinson and Rollin 2007; Rollin et al. 2007; Sanchez et al. 2004).

TABLE 2-4 Symptoms of Ebola Virus-Infected Humans

Clinical Symptom	Frequency Observed in Survivors (%)	Frequency Observed in Fatal Cases (%)
Abdominal pain	68	62
Abortion	5	2
Anorexia	47	43
Anuria	0	7
Arthralgia or myalgia	79	50
Asthenia	95	85
Bleeding from puncture sites	5	8
Bleeding from the gums	0	15
Bloody stools	5	7
Chest pain	5	10
Conjuctival infection	47	42
Convulsions	0	2
Cough	26	7
Diarrhea	84	86
Dysesthesia	5	0
Epistaxis	0	2
Fever	95	93
Headaches	74	52
Hearing loss	11	5
Hematemesis	0	13
Hematoma	0	2
Hematuria	16	7
Hemoptysis	11	0
Hepatomegaly	5	2
Hiccups	5	17
Lumbar pain	26	12
Maculopapular rash[a]	16	14
Melena	16	8
Nausea and vomiting	68	73
Petechiae	0	8
Sore throat, odynophagia, or dysphagia	58	56
Splenomegaly	5	2
Tachypnea	0	31
Tinnitus	11	1

[a] variable detection may be attributed to skin color
SOURCE: Adapted from Bwaka et al. 1999.

TABLE 2-5 Symptoms of Bundibugyo Virus-Infected Humans

Clinical Symptom	Frequency Observed in Survivors (%)	Frequency Observed in Fatal Cases (%)
Abdominal pain	88	93
Anorexia or weight loss	83	80
Arthralgia or myalgia	83	86
Diarrhea	92	87
Difficulty breathing	26	57
Fatigue	96	100
Fever	100	100
Headaches	84	93
Hiccups	17	40
Maculopapular rash[a]	35	33
Nausea and vomiting	92	87
Sore throat, odynophagia,or dysphagia	43	60

[a] variable detection may be attributed to skin color
SOURCE: Adapted from MacNeil et al. 2010.

Experimental Filovirus Infection in Animals

The animals currently used in experimental filovirus research are mostly nonhuman primates and rodents (see Table 2-6). The majority of published data from well-established animal models,[8] including detailed data on pathogenesis and pathology of disease from African green and rhesus monkeys and cynomolgus macaques, stem from experiments with EBOV or MARV strains (Ebola virus references: Alves et al. 2010; Baskerville et al. 1978, 1985; Bowen et al. 1978; Bray et al. 1998; Connolly et al. 1999; Dadaeva et al. 2006; Geisbert 2003a,b; Jaax et al. 1996; Johnson et al. 1995; Kolesnikova et al. 1997; Pereboeba 1993; Ryabchikova et al. 1993, 1996a, 1998, 1999a, 2004; Vogel et al. 1997; Marburg virus references: Bechtelsheimer et al. 1970; Haas et al. 1968a,b; Korb and Slenczka 1971; Lub et al. 1995; Murphy et al. 1971; Oehlert 1971; Robin et al. 1971; Ryabchikova et al. 1994, 1996b, 1999b; Simpson 1969; Simpson et al. 1968; Warfield et al 2007; Zlotnik 1971; Zlotnik and Simpson 1969). Table 2-7 compares hematological disturbances and mean time to death observed in various nonhuman primate species following EBOV infection. With the possible exception of the hematological responses, nonhuman primates infected with MARV or EBOV roughly reflect the human disease, without significant contradictions between clinical signs and gross pathology.

[8] "Well-established" refers to animal models that are in use in several BSL-4 facilities and are referred to repeatedly in publications on animal use in filovirus research.

TABLE 2-6 Animals Used for the Development of Animal Models for Filovirus Disease

Virus	Animal	Status of Model
Marburg virus (MARV)	Common marmoset (*Callithrix jacchus*)	Model under evaluation, supposedly lethal, unpublished
	African green monkey (*Chlorocebus aethiops*)	Well-established lethal model, published
	Common squirrel monkey (*Saimiri sciureus*)	Anecdotal lethal "model," uncharacterized, unpublished
	Rhesus monkey (*Macaca mulatta*)	Well-established lethal model, published
	Dunkin Hartley and strain 13 guinea pigs	Well-established lethal model (requires virus adaptation), published (strain 2 guinea pigs are sometimes also used but their pathology has not been described in detail)
	Syrian (golden) hamsters	Historical lethal model (requires virus adaptation), basically uncharacterized
	BALB/c and SCID BALB/c laboratory mice	Recently established model, lethal (requires virus adaptation), published
Ravn virus (RAVV)	Cynomolgus macaque (*Macaca fascicularis*)	Uncharacterized model, mentioned in publications
	Rhesus monkey (*Macaca mulatta*)	Established lethal model, published
	BALB/c and SCID-BALB/c laboratory mice	Recently established model, lethal (requires virus adaptation), published
Bundibugyo virus (BDBV)	Cynomolgus macaque (*Macaca fascicularis*)	Model under evaluation, lethal
	Rhesus monkey (*Macaca mulatta*)	Model under evaluation, thus far unsuccessful, unpublished
Taï Forest virus (TAFV)	Cynomolgus macaque (*Macaca fascicularis*)	Established partially lethal model, published
	Rhesus monkey (*Macaca mulatta*)	Model under evaluation, no data available
Reston virus (RESTV)	Cynomolgus macaque (*Macaca fascicularis*)	Well-established model, infrequently lethal, published
	Domestic pig (*Sus scrofa*)	Model under evaluation, no data available
	African green monkey (*Chlorocebus aethiops*)	Not well-established model, often nonlethal, published
Sudan virus (SUDV)	African green monkey (*Chlorocebus aethiops*)	Not well-established model, lethal, published
	Cynomolgus monkey (*macaca fascicularis*)	Established lethal model, published
	Rhesus monkey (*Macaca mulatta*)	Not well-established model, lethal, published

	ICR laboratory mice	Anecdotal lethal "model," uncharacterized, unpublished
Ebola virus (EBOV)	Common marmoset (*Callithrix jacchus*)	Novel lethal model
	Cynomolgus macaque (*Macaca fascicularis*)	Well-established model, lethal, published
	African green monkey (*Chlorocebus aethiops*)	Well-established model, lethal, published
	Hamadryas baboon (*Papio hamadryas)*	Well-established model, lethal, published
	Rhesus monkey (*Macaca mulatta*)	Well-established model, lethal, published
	Domestic pig (*Sus scrofa*)	First experiments published, but lethality unclear
	Dunkin Hartley and strain 13 guinea pigs	Well-established model, lethal, (requires virus adaptation), published (strain 2 guinea pigs are sometimes also used but their pathology has not been described in detail)
	Syrian (golden) hamsters	Model under evaluation (requires virus adaptation), supposedly lethal, unpublished
	BALB/c, C57BL6, and ICR laboratory mice	Well-established model, lethal, (requires virus adaptation), published

SOURCE: Adapted from Kuhn 2008 and references therein.

TABLE 2-7 Animal-Specific Hematological Differences in Nonhuman Primate Models of Ebola Virus Disease, Infected with 1-10 LD_{50}

Animal	Mean Time to Death	Hematological Disturbance
Cynomolgus macaque (*Macaca fascicularis*)	10-14 days	Fibrin depositions
African green monkey (*Chlorocebus aethiops*)	7-8 days	Microcirculatory disturbances (capillary stasis, erythrocyte aggregation), organs engorged with blood, no hemorrhage, no fibrin depositions
Hamadryas baboon (*Papio hamadryas)*	9-10 days	Erythrocyte diapedesis
Rhesus monkey (*Macaca mulatta*)	7-8 days	Fibrin depositions, prominent hemorrhages

SOURCE: Adapted from Kuhn 2008.

Table 2-8 compares the clinical signs of various EBOV-infected animal species with those of infected humans and presents a rather well-characterized collection of animal models of filovirus infection.

TABLE 2-8 Comparison of Data from Ebola Virus Animal Models with Data from Humans

Symptom	Mice (postvirus adaptation)	Guinea Pigs (postvirus adaptation)	Nonhuman Primates	Humans
Disease duration to death (days)	4-55	6-12	5-10	3-30
Virulence	High	High	High	High
Fever	No	Yes	Yes	Yes
Peak viremia (plaque-forming unit per milliliter)	7.5×10^7-5.6×10^{11}	$> 0^{5.2}$	10^6-10^8	$10^{6.5}$
Hemorrhages	Variable	Rare	Dependent on primate type	Occasional
Maculopapular rash	No	No	Dependent on primate type	Variable (detection often depends on skin color)
Disseminated intravascular coagulation	no	Data conflicting	Yes	Yes
Liver enzymes	Elevated	Elevated	Elevated	Elevated
Lymphopenia	Unknown	Yes	Yes	Yes
Lymphocyte apoptosis	Yes	Unknown	Yes	Yes
Thrombocytopenia	Yes	Yes	Yes	Yes
Cytokine response	Yes	Unknown	Yes	Yes
Nitric oxide level elevation	Unknown	Unknown	Yes	Yes

SOURCE: Adapted from Kuhn 2008 and references therein.

Although the time to death for humans extends beyond that of the copresented animal species, it is probably affected by a number of external factors (e.g., whether a patient was hospitalized or received any other care). The extended range of time to death in mice is a characteristic of the EBOV mouse model proposed by Bray and colleagues (1998). In more recent studies, MARV-infected mice die 7-10 days postinfection (Warfield et al. 2009), which is closer to the time of death of human patients. Despite these data, there is currently no consensus in the field on which nonhuman primate model better approximates the course of human infection, in part because of the paucity of cytokine data from the various nonhuman primate models that could be compared with the human data collected in the studies mentioned above. Specifically, biochemical analysis of blood from EBOV-infected cynomolgus macaques and rhesus monkeys revealed increased concentrations of IL -6, whereas IL-2 and IL-10 were

rarely detectable (Hensley et al. 2002). A different study using RESTV, rather than a clinically relevant filovirus known to infect humans, revealed a different cytokine activation profile than that shown in human EBOV or SUDV infections (Hutchinson et al. 2001). It is also important to note that RESTV, one of two filoviruses that thus far are thought apathogenic in humans, is virulent in cynomolgus macaques, but not in African green monkeys. The results in cynomolgus macaques raise the question of whether they are indeed valuable heterotypic approximations of humans, given that they should succumb only to EBOV but not to RESTV.

To date, five filoviruses (MARV, RAVV, BDBV, EBOV, and SUDV) are being studied for countermeasure development. Although some of the animal models for the most commonly studied of those viruses, MARV and EBOV, are well established and published, data from animal experiments with the other three have not yet been satisfactorily evaluated for studies of pathogenesis or evaluation of pharmaceuticals or vaccines. Moreover, it is apparent that rodents are not good approximations for human disease for the following reasons: (1) the virus needs to be genetically altered (adapted by serial passage) before it is administered to the animals so that they will succumb; (2) disseminated intravascular coagulation (DIC), which is a prominent symptom of infected humans, does not seem to be a hallmark symptom of their disease; and (3) the typical maculopapular rash is absent.

TULAREMIA

The development of animal models for tularemia is interesting because of the availability of both clinical information regarding direct challenge into humans and data regarding the efficacy of the current investigational new drug (IND) vaccine Live-Vaccine Strain (LVS) to protect human volunteers against direct pulmonary challenges with virulent strain Schu S4 of *Francisella tularensis* (Hornich and Eigelsbach 1966; McCrumb et al. 1957; Saslaw and Carlisle 1961). Consequently, endpoints (diagnostic and clinical) are available that can be used to judge the worthiness and relevance of a tularemia animal model and possibly refine the experiments in this line of research. On the basis of these data, any comparable animal model would be expected to be (1) very sensitive to infections with Schu S4 (Biovar A) serotypes of *Francisella*; (2) resistant to infection by high doses of the LVS; and (3) protected from significant morbidity and mortality by prevaccination with LVS.

Nonhuman primates and mice are the most prevalent animal models for primary pulmonary tularemia. Laboratory mice have been extremely useful for dissecting the immune response to *F. tularensis* and understanding some of the pathophysiology (Coriell et al. 1947; Downs et al. 1949; Ruchman and Foshay 1949). Indeed, the pathology of pyrogranulomae and the primary organ involvement of lung, spleen, and liver are consistent between humans and laboratory mice. However, unlike the human, mice are sensitive to LVS infection, and low doses of LVS do not reproducibly protect these animals from subsequent challenge by Schu S4 (Conlan et al. 2003; Wu et al. 2005); these facts diminish the use of this model for vaccine development. Recently a model based on Fischer 344 rats was shown to be resistant to LVS administration. Further, vaccination with LVS by any route protects these animals against subsequent challenge with relatively high doses of Schu S4 (Wu et al. 2009).

The nonhuman primate model for pulmonary tularemia exhibited similar pathology to that of humans in the course of primary infection, while LVS administration elicited a strong protection against challenge with the Schu S4 strain (Lyons and Wu 2007). If these nonhuman primate models are reproducible, then it is possible that vaccines against *F. tularensis* could be developed. However, little work has been done to decipher the basic mechanism of protection and immunity in these animals and to determine absolute or relative immune responses as correlates of protection. Because of limited understanding of how the human cellular responses develop antibacterial defenses, it remains hard to

develop correlates of protection in humans not only to predict clinical benefits but also to increase confidence in the protection afforded by vaccination. If correlates of protection are known, they may further help advance the research to determine an "effective dose" in humans based on animal experimentation, which is a required element of the Animal Rule.

ANTHRAX[9]

The challenges facing the production of countermeasures may be highlighted by a discussion of the process applied to the biothreat posed by *Bacillus anthracis*. *B. anthracis* has been studied for decades, and the details related to the life cycle of the bacterium are well known (Hugh-Jones and Blackburn 2009); therefore, the development of new products for treatment is expected to be straightforward. The aerosolization of *B. anthracis* spores is the greatest biothreat risk associated with this pathogen, as pulmonary anthrax is the most lethal form of the disease. Once the spores are inhaled, they are phagocytosed by alveolar macrophages and taken to local lymph nodes where they germinate and disseminate as vegetative bacilli to surrounding tissues via the bloodstream. The timing of this dissemination is unpredictable because it depends on the generation of virulence factors, such as the capsule, which engulfs and protects the bacilli, and the intracellular constitution of the tripartite anthrax toxin. The role of these factors has been well described (Makino et al. 2002; Moayeri and Leppla 2004).

Although the rabbit and many nonhuman primate species are considered the primary animal models for therapeutic product development against *B. anthracis*, a lot of information has been collected through studies in rodent models. Laboratory mice have always been an attractive model because of (1) the plethora of available tools to dissect the host responses that develop against the aerosol challenge with *B. anthracis*; (2) their small "footprint" and necessary housing area; and (3) the minimal costs associated with their procurement, care, and use. The murine repertoire of antibodies and T-cell reactivity in response to *B. anthracis* challenge is generated in a process very similar to that of humans. Across several *B. anthracis* studies in laboratory mice, the primary difference with the human disease is the dominant virulent factor, which in mice is the capsule (Chand et al. 2009). Encapsulated strains of *B. anthracis* that do not express toxin remain virulent and lethal in most murine models of anthrax except for the susceptible A/J strain. A/J mice deficient in complement protein C5 die from a toxin-mediated death following infection with low doses of the nonencapsulated Sterne strain (Welkos and Friedlander 1988). In this animal model, where the toxin is the target, the current Anthrax Vaccine-Adsorbed (AVA) vaccine provides robust protection, as do other antitoxin modalities, such as antiserum to recombinant protective antigen (Pitt et al. 2001). On the basis of the limited role for *B. anthracis* toxins in the infection of laboratory mice, these animals are considered a poor model for human anthrax, whose pathogenesis depends on the virulence of toxin (Heninger et al. 2006).

The rat model is thought to be inadequate because of the high baseline resistance of these animals to infection with *B. anthracis* spores. Some strains of rats (e.g., Fischer 344), however, display high sensitivity to injected purified toxin and are therefore routinely used to screen antitoxin candidates, such as human monoclonal antibodies (Beall and Dalldorf 1966; Sawada-Hirai et al. 2004). Guinea pigs were used in some seminal studies to describe the trafficking of spores delivered via the lung before dissemination (Ross 1957). Guinea pigs are used in potency assays for the licensed AVA

[9] The National Institute for Allergy and Infectious Diseases' efforts to fund research into the standardization of biodefense-related animal models for product development under the Animal Rule deserve credit for advancing the anthrax model in particular and for raising awareness of all models more generally (see Appendix C).

vaccine based on the protection observed following challenge with parenterally administered spores (FDA 1973). The understanding of the efficacy of this vaccine dates back to data derived from vaccinated workers in wool processing plants in the 1950s (Brachman et al. 1962). Analysis of these data coupled with the fact that guinea pigs challenged by aerosolized anthrax spores are not reliably protected by the AVA vaccine (Fellows et al. 2001) demonstrate that this animal model is not optimal for vaccine testing and screening. Such a priori knowledge of the expected efficacy of a vaccine in humans is unlikely to be available for the majority of current biothreats.

Rabbits and nonhuman primates, such as rhesus monkeys, are sensitive to pulmonary anthrax and demonstrate many of the pathological findings observed in humans (Vasconcelos et al. 2003; Zaucha et al. 1998). Moreover, the gross lesions seen in the cynomolgus macaque pulmonary anthrax model are similar to those seen in infected humans, including splenomegaly, lymph node enlargement, and hemorrhages in several different organs. Mediastinitis was observed in approximately 30% of the infected animals (Vasconcelos et. al. 2003). As both rabbit and macaque species are well-protected by the AVA vaccine, they have been very useful in the development of prophylactic therapeutics against anthrax (Phipps et al. 2004).

In contrast to the nonhuman primate models, the rabbit provided few, if any, clues to the disease progression. Thus, it has been challenging to develop a reproducible rabbit model for therapeutics to be administered during the dissemination stage of the disease for the following reasons: (1) the rabbit shows very few to no clinical symptoms postinfection, thus the timing for postinfection intervention is not easily discernible; and (2) due to the unpredictable timing of dissemination from the lung into the bloodstream, each animal may need therapeutic intervention at different time points. Because the time from infection to death typically occurs within 48-72 hours, this experiment presents enormous logistical challenges.

Although the rabbits and nonhuman primates appear to be the best models for medical countermeasure development under the regulatory provisions of the Animal Rule, their use poses significant challenges with regard to housing capacity, number of animals needed for statistically meaningful results, and cost of procurement and care. Moreover, societal sensitivities toward the use of nonhuman primates in research pose additional impediments to the continued use of these animals in the development and production of medical countermeasures for emerging infections and biothreats.

LESSONS LEARNED FROM DEVELOPING ANIMAL MODELS FOR THERAPEUTIC PURPOSES AGAINST BIOTHREAT AGENTS

The search for prophylactics and therapeutics against infection of *B. anthracis*, as summarized in the previous pages, has encountered a number of hurdles, some of which stem from trying to force the animal model to fit the experimental protocol instead of selecting the most appropriate model based on the desired experimental outcome (for an expanded discussion on this issue see Chapter 4, page 56). Furthermore, the process of developing animal models and medical countermeasures has been intimately linked in such a manner that the development and subsequent fitness of the model is determined solely in the context of the countermeasure rather than in a product-neutral fashion. It is important to realize that the value of animal models depends on the context of the scientific question to be investigated.

A number of currently used animal models do not translate well to the human condition. Furthermore, most models are complex and therefore costly to develop. High levels of biocontainment

are necessary to safely perform research with these pathogens, and additional restrictions are imposed by their classification as Select Agents.[10] These facts coupled with the large numbers of animals necessary for the research and development of countermeasures point to the need to reevaluate the ways these models are developed and used. It would be more beneficial to develop models with a broader application profile that can be used to develop more than one countermeasure.[11] Such an approach might address not only the conundrum of over-relying on analogous systems to predict efficacy of products in humans, but may be of significance in an encounter with an "unknown-unknown".

As previously stated, one of the potential unintended consequences of the Animal Rule is the ambitious expectation that animal efficacy studies predict the human condition. This expectation is daunting for two reasons: (1) there is not enough primary data from humans to which animal data can be compared, and (2) the ability of animal models to reflect the human disease is not absolute. As discussed on page 19, the collection of more detailed clinical data for filovirus infections has been attempted multiple times in the past and failed. At this time, there is no reason to believe that collection of data from filovirus disease outbreaks may improve in the immediate future. Further, while potentially more detailed human data on tularemia and anthrax exists, it is far from comprehensive. **The animal models currently available may be the only avenue to accrue some data on pathogenesis, perhaps on correlates of protection, and, through that, on efficacy of pharmaceuticals or vaccines**. These circumstances also reflect the TMT's other concern, namely, the deliberate attack on warfighters with an "unknown-unknown," that is, an agent for which human clinical data are not available at the time of attack. Therefore, the collection of human clinical data is of utmost importance in order to verify the usefulness and augment the strengths of available models.

The previous sections described the variable results obtained by using animals belonging to different species, a fact also encountered in other fields of biomedical research (e.g., see Craig 2009; Mogil 2009). In addition to factors such as host susceptibility and clinical pathology, the progression of the disease in the different animal species may not resemble that of humans, possibly resulting in failed translational efforts, as evidenced by the increase in attrition rates of products in the later stages of clinical development. In a recent meta-analysis of the potential reasons that animal experiments fail to translate into clinical trials, van der Worp and colleagues (2010) identified recurring themes across animal studies that may prevent them from providing a "correct basis for generalizations to the human condition" as represented in clinical trials (what the authors define as external validity: "the extent to which the results of an animal experiment provide a correct basis for generalizations to the human condition", p 3; see Table 2-9).

Table 2-9 presents important and common methodological deficiencies, some of which are further discussed in Chapter 5. An additional consideration may be the choice of animals that are young and otherwise healthy, whereas the human patients may have co-morbidities (van der Worp et al. 2010). Addressing some of these issues may be as simple as thoroughly studying the literature. As elaborated further in Chapter 5, however, systematic sharing of data with the wider research community will improve the predictive capacity of animal models. In summary, the more approximation exists between the animals and the conditions under which they are used in efficacy

[10] "Select Agents" and toxins are agents that the Department of Health and Human Services "considers to have the potential to pose a severe threat to human health. A list of these agents are found in the Select Agents regulation (42 CFR 73)." See http://www.selectagents.gov/FAQ_General.html#sec1q3.

[11] The platform technology approach adopted by the TMT fits well with the product-neutral approach. As further discussed in Appendix C, the National Institute of Allergy and Infectious Diseases (NIAID) is similarly focused on product-independent and product-dependent (i.e., product-neutral) models until such time as the product is ready for the final efficacy studies (also known as pivotal studies).

studies and the characteristics of the human population for which the countermeasures are intended (including clinical status), the better the chances that the countermeasure would be successful.

TABLE 2-9 Common Causes of Reduced External Validity of Animal Studies

- Assessment of the effect of a treatment in a homogeneous group of animals versus a heterogeneous group of patients.
- The use of either male or female animals only, whereas the disease occurs in male and female patients alike.
- The use of models for inducing a disease or injury with insufficient similarity to the human condition.
- Delays to start of treatment that are unrealistic in the clinic; the use of doses that are toxic or not tolerated by patients.
- Differences in outcome measures and the timing of outcome assessment between animal studies and clinical trials.

Source: Adapted from van der Worp et al. 2010.

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3

Ethical and Regulatory Challenges in the Development of Countermeasures

The Committee on Animal Models for Assessing Countermeasures to Bioterrorism Agents heard many comments during the open-meeting sessions (see Appendix D) about the effects of the Animal Rule on the research and development of countermeasures. It is not, however, within the Committee's purview to evaluate this law. The 2010 Public Health Emergency Medical Countermeasures Enterprise review, spearheaded by the Department of Health and Human Services, has identified a number of priorities for the Food and Drug Administration (FDA), including to "examine the current constraints posed by the Animal Efficacy Rule and identify strategies to improve its implementation" (DHHS 2010, p 11). Accordingly, this chapter presents the legal history of the Animal Rule and discusses some of the ethical challenges associated with the development of countermeasures, such as public information and disclosure of facts relating to products approved under the Animal Rule, and issues of informed consent. A short discussion of the two products approved to date under the Animal Rule is also offered, as well as a short description of the FDA's drug development regulatory process.

HISTORY OF THE ANIMAL RULE AND ETHICAL CONSIDERATIONS

To respond effectively to potential biological and chemical threats, drugs and biological products are being developed and produced for which it is neither ethical nor legal to conduct efficacy studies with humans because of the unacceptably high risk of harm that testing itself would pose. The need to produce such countermeasures and the constraints imposed by their research are the underlying rationale for the FDA's Animal Model Rule (Animal Rule; 21 CFR Parts 314 and 601[2002]; see Appendix A).

With respect to the products for which it has oversight, the FDA's statutory mission includes the provision that the agency "shall protect the public health by ensuring that human...drugs are safe and effective" (21 USC § 393(b)). In addition to its crucial public health role in preventing the distribution of unsafe and nonbeneficial substances, the FDA is also responsible for approving new drugs under the authority of the Public Health Service Act (42 USC 201 et seq.). In this role, the FDA is charged with "helping to speed innovations that make medicines more effective, safer, and more affordable; and

helping the public get the accurate, science-based information they need to use medicines and foods to maintain and improve their health" (FDA 2010). This multifaceted charge shapes the FDA's responsibility for the use of investigational new drugs (INDs) developed pursuant to the Federal Food, Drug, and Cosmetic (FFDC) Act, as amended by the Food and Drug Administration Modernization Act of 1997, 21 USC 301 et seq. (see 21 CFR Part 312), and the approval of new indications for previously approved substances.

During the 1990-1991 Persian Gulf War, the FDA granted waivers to the Department of Defense (DoD) for the off-label administration of pyridostigmine bromide (PB) tablets for prophylaxis against nerve agent and botulinum toxoid vaccine for prophylaxis against botulism to military personnel without research informed consent on the basis of 21 CFR Part 50 [1997]. In 1999, the FDA recognized that it would be contrary to the public interest and inconsistent with the public health purpose of the Public Health Service Act to conclude that a drug or biological product could not be approved because human efficacy trials could not be ethically or legally conducted (21 CFR Parts 314 and 601; FDA 1999). Therefore, the FDA recommended that when human efficacy trials could not be done ethically or legally, rather than leave its evaluators without a basis upon which to "fairly and responsibly" (FDA 1999, p 53964) conclude that a drug or biological product would be effective, animal studies could provide sufficient information to support a finding of "substantial evidence" that would warrant approval (p 53965). The agency suggested that it would approve a "new drug or biological product on the basis of adequate and well-controlled animal trials when it is scientifically reasonable to expect that the effect of the drug or biological product in animals is reasonably likely to predict clinical benefit in humans" (p 53964).

The FDA proposed amending its regulations to identify the information necessary to provide sufficient evidence of the "efficacy of new drug and biological products used to reduce or prevent the toxicity of chemical, biological, radiological, or nuclear substances when adequate and well-controlled efficacy studies in humans cannot be ethically conducted because they would involve administering a potentially lethal or permanently disabling toxic substance or organism to healthy human volunteers without a proven treatment and field trials (assessment of use of the product after accidental or hostile exposure to the substance) are not feasible" (FDA 1999, p 53961). It advised that in such situations "certain new drug and biological products that are intended to reduce or prevent serious or life-threatening conditions could be approved for marketing based on evidence of effectiveness derived from appropriate studies in animals, without adequate and well-controlled efficacy studies in humans" (p 53961). Safety still would be studied in human volunteers after approval and the products "would be expected to provide meaningful therapeutic benefits to patients over existing treatment" (p 53963). The agency acted under the authority of the FFDC Act in proposing amendments to 21 CFR Part 314 Subpart I (Approval of New Drugs for Use Against Lethal or Permanently Disabling Toxic Substances When Efficacy Studies in Humans Ethically Cannot Be Conducted) and Part 601 Subpart G (Approval of Biological Products for Use Against Lethal or Permanently Disabling Toxic Substances When Efficacy Studies in Humans Ethically Cannot Be Conducted).

Three years later, in 2002, the final rule provided for "approval of certain new drug and biological products based on animal data when adequate and well-controlled efficacy studies in humans cannot be ethically conducted because the studies would involve administering a potentially lethal or permanently disabling toxic substance or organism to healthy human volunteers and field trials are not feasible prior to approval. Under this rule, in these situations, certain new drug and biological products that are intended to reduce or prevent serious or life-threatening conditions can be approved for marketing based on evidence of effectiveness derived from appropriate studies in animals, without adequate and well-controlled efficacy studies in humans" (FDA 2002, p 37989).

The FDA Center for Biologics Evaluation and Research (CBER) and the FDA Center for Drug Evaluation and Research (CDER) were charged with evaluating, approving for efficacy, and regulating drugs and biological products under the Animal Rule while "the final determination that it is unethical to conduct studies in humans [would] be made by the reviewing officials in [the] FDA." If possible, the agency would consult with an advisory committee during the approval process (FDA 2002, p 37992]).[12]

To date, the FDA has approved two drugs under the Animal Rule. On February 5, 2003, utilizing the Animal Rule for the first time, the FDA approved PB for combat use by U.S. military personnel to increase survival after exposure to soman "nerve gas" poisoning (FDA 2003a). On December 15, 2006, the agency approved Cyanokit (containing the drug hydroxocobalamin, a natural form of vitamin B_{12}) for the treatment of known or suspected cyanide poisoning (FDA 2006a; for additional information, see p 44).

Public Information and Disclosure

Because the Animal Rule combines intervention with simultaneous collection of safety data (e.g., "…field studies, to verify and describe the drug's [or biological product's] clinical benefit and to assess its safety when used as indicated…such postmarketing studies would not be feasible until an exigency arises", p 37995 and 37997) the use of products approved under it requires special disclosure. The Animal Rule emphasizes the importance of advising recipients that a product approved pursuant to the Animal Rule had not been "studied for efficacy in humans because of ethical or feasibility reasons" (FDA 2002, p 37990). It further requires product labeling "in language that is easily understood" to be available with the dispensing or administration of the product "if possible" (p 37992).

Ongoing controversy about the effects of the DoD's previous use of off-label drugs and vaccines (Connoly 2001a; Golomb 2008; Grady 2004; Miller 2002; Parmet 2010; Rettig 1999; SteelFisher et al. 2010), coupled with popular theories about the sources of new diseases and the utility of drugs and vaccines, suggests that the administration of drugs and vaccines approved under the Animal Rule could prompt a backlash against the FDA and the DoD precisely at the time that effective countermeasures would be most needed. In the past, the DoD's targeted health education campaigns and efforts at comprehensive communication about prophylaxis against and treatment of weaponized diseases have been met with mistrust (Miller 2002; MVRD 2011; Rettig 1999). Thus, in response to a bioterroristic event or the use of bioweapons, medical uncertainty may easily give way to anxiety, fear, and panic among the public, both about the weaponized disease and about the means proposed to address it, with the concomitant refusal to be vaccinated and/or take drugs posing a threat to public health and safety.

Accordingly, educational plans and disclosure strategies would be necessary to address not only these concerns but also the US public's demand for unapproved or off-label-use drugs for when the perceived threat of disease, disability, or death is high. Research ethicists routinely observe the "therapeutic misconception", under which study participants and investigators alike presume that clinical investigation inherently offers direct benefits to participants (Appelbaum et al. 1987). Nationally, demand for the treatment of HIV and AIDS in the 1990s and of advanced cancers to this date has shifted the ethical debate about justice away from the protection of human participants from research risks to focus on the participants' fair access to clinical trials (London et al. 2010). A perceived medical crisis, such as a bioterroristic attack, could easily create an undue and arguably inappropriate public demand for drugs, vaccines, or other biological products under development or approved under

[1] For example, during a public health emergency such a consultative process might not be feasible.

[2] An FDA advisory committee opined on the first application for approval of a novel product under the Animal Rule in October 2009 (see section *Newer Products under the Animal Rule*).

the Animal Rule, even for products developed solely for combat use. For example, the 2001 mailings of *B. anthracis* prompted panic buying and hoarding of Ciprofloxacin and an increased civilian interest in the DoD's approved anthrax vaccine, even as controversy grew about the vaccine's mandatory military use and whether the vaccine was effective against inhalation anthrax (Annas 2005; Connolly 2001b; Miller 2002). More recently, during the H1N1 pandemic of 2009-2010, critics protested shortages, allegedly unfair priorities and geographic distribution of vaccines and antiviral medication at the same time when others expressed suspicion of the speed with which a vaccine was developed, tested, and produced and states debated mandatory vaccination plans (Parmet 2010).

The FDA public information program and specific disclosures for drugs and vaccines approved under the Animal Rule will play a key role in the DoD's plans for responding to the threat of bioweapons and bioterrorism. The DoD's plans will need to be consistent with approved indications for the administration of the drugs and vaccines. Indeed, advising not only the individuals targeted for the receipt of new drugs and vaccines but also the general population, civilian as well as military, is critical for the acceptance and, therefore, success of any product approved under the Animal Rule. Public education about the real and projected threat of bioterroristic agents and the development of countermeasures is a crucial step in preparing at-risk groups to receive more specific information if intervention becomes necessary.

The Unresolved Issue of Informed Consent

As Richard Rettig pointed out in 1999, the use of investigational drugs and vaccines in response to chemical and biological weapons is not easily classified as either clinical treatment or research. The use of off-label or newly developed drugs or biologics is traditionally permissible only under a formal research protocol, but their use as countermeasures would have a primarily therapeutic rather than a research purpose. This distinction is further blurred when the goal is prophylaxis against an anticipated threat rather than an emergency response to an actual exposure or infection. Furthermore, unlike the off-label use of past countermeasures, drugs and vaccines developed for use in response to bioweapons and biothreat agents and approved under the Animal Rule probably will not have established prior uses in other contexts. This fact will almost certainly generate public concern about any attendant risks. The DoD's waiver of informed-consent requirements for combat use of PB and botulinum toxoid vaccine became a lightning rod for controversy in the 1990s (Rettig 1999, 2000), even though their use fell within the battlefield exceptions to informed consent requirements for INDs (e.g., 21 CFR 50.23). The DoD's 1998 mandatory administration of anthrax vaccine to 2.4 million service members sparked further debate that persists today (Annas 2002, 2010; Connolly 2001a,b; Miller 2002; MVRD 2011).

In standard usage, "informed consent to treatment" refers to the process in which a physician proposes an intervention (drug, device, or procedure) to a patient of record that, in the physician's professional judgment, would serve the patient's specific medical interests. The physician explains (1) the nature of the proposed intervention; (2) its likely consequences, including anticipated benefits and risks of harm; and (3) the reasonable alternatives, including forgoing treatment, and their benefits and risks. The patient is then free to accept or refuse the proposed intervention. Although legal requirements call for variable levels of detail in required disclosures, depending on the nature of the intervention and its attendant risks and anticipated benefits, the ethical grounds for informing the patient and seeking his or her consent extend to all treatment (Beauchamp and Childress 2001; Faden and Beauchamp 1986).

By contrast, the standard usage of "informed consent to research" refers to the process in which an investigator (often but not always a physician) recruits an individual to undergo a new or modified intervention "as part of a systematic investigation designed to develop or contribute to generalizable

knowledge" (45 CFR 46.102(d)).[3] The intervention may or may not serve the specific interests or needs of the individual, but meeting those needs and interests is secondary to gathering data through a standardized protocol designed to answer the research question. The investigator must explain to the participant (1) the nature of the proposed intervention; (2) its likely consequences, including anticipated benefits and risks of harm; (3) reasonable alternatives, including not participating in the investigation, and their risks and potential benefits; and (4) the voluntary nature of participation and the individual's right to leave the study at any time. The individual is free to accept or refuse participation.

The standards of informed consent in research contexts, including required documentation, are more stringent than those in treatment contexts because (1) the risks and unknowns of research are greater than those of established treatments, and the anticipated benefits are less well known; and (2) the specific interests of the individual undergoing the intervention are secondary to the goals of the study. Nonetheless, in both contexts, informed consent is predicated upon the individual's understanding and voluntariness. In public health crises, such as disease outbreaks or bioterrorism events, it may not be possible to obtain individual consent for treatment.

The absence of a clearly articulated legal and ethical framework opens the door for renewed confusion and conflict over the DoD's (or other government agencies') authority to administer new drugs and biologics that have not been previously tested on humans for their efficacy and were developed through the use of animal models to prevent or treat life-threatening diseases resulting from bioweapons or biothreat agents. Furthermore, in light of the complex historical debate about the ethical and legal grounds for waiving consent requirements in treatment and research settings, the use of the Animal Rule creates a pressing need to clearly define when and why new drugs and vaccines are investigational and the criteria by which consent requirements for their use may be waived. As discussed in the previous section, public information and disclosure about informed-consent procedures for dispensing or administration of these products will be crucial to the public understanding and acceptance of the Animal Rule and the drugs and biologics approved under it.

Draft Guidance and the Animal Rule

On January 21, 2009, in accord with the agency's Administrative Practices and Procedures/Good Guidance Practices regulation (21 CFR 10.115 [2000]), the FDA issued for comment the "Draft Guidance for Industry: Animal Models—Essential Elements to Address Efficacy Under the Animal Rule" (FDA 2009a). The draft was prepared by the Animal Model Characterization Working Group in CDER in cooperation with CBER. The draft announced that "when human efficacy studies are neither ethical nor feasible, animal efficacy studies may be relied on under the Animal Rule to support approval or licensure of a drug or biological product. This guidance identifies and discusses the critical characteristics of an animal model" (FDA 2009a, p 3610). Comments were requested for consideration before the agency began work on the final guidance. To date the guidance remains in use as interpretive only. As noted in the announcement for comment "this draft guidance, when finalized, will represent the [agency's] current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public" (p 3610).

[3] On July 26, 2011 the Department of Health and Human Services issued an advanced notice of proposed rulemaking for revisions to the current human subjects research regulations. These would impact the "Common Rule", i.e., 45 CFR Part 46 Subpart A, and potentially the FDA's regulations governing human subjects research, i.e., 21 CFR Parts 50, 56, 312, and 812. See http://www.federalregister.gov/articles/2011/07/26/2011-18792/human-subjects-research-protections-enhancing-protections-for-research-subjects-and-reducing-burden#p-20.

The absence of a final guidance makes the evaluation and enforcement of the use of the Animal Rule problematic. Under these circumstances, any consistent engagement with research that could expedite the development of drugs and biological products is discouraged; for instance, the sharing of data to avoid known fruitless studies and repetition of completed work; to yield better models; to lower costs; and to reduce the number of animals (including the numbers of nonhuman primates) with the concomitant reduction in animal pain, distress, and deaths.

THE LOW APPROVAL RATE TO DATE UNDER THE ANIMAL RULE

The Animal Rule Has Been Used Rarely and for No Novel Products

To date, the Animal Rule has been used only twice to approve new products. The countermeasures were not novel in either case; one approval was for a new clinical indication of an already approved drug, and the other approval was for a product that was in use in France.

As noted above, the first countermeasure approved under the Animal Rule was pyridostigmine bromide (PB) in 2003. PB was indicated for pretreatment of exposure to the nerve agent soman. Since a different dose of the drug had previously received the FDA's approval for treating myasthenia gravis in 1955, the Animal Rule was not used for a novel compound but to extend the indicated use of an already existent drug as a countermeasure (Gronvall et al. 2007). The second approval, in late 2006, was for Cyanokit (FDA 2006a). This drug is indicated for the treatment of known or suspected cyanide poisoning as a result of terrorism or smoke inhalation. Hydroxocobalamin (i.e., vitamin B_{12}), the ingredient in Cyanokit, was approved in France in 1996 and was available in the United States at a much lower dose (Gronvall et al. 2007). There was already human data available to indicate that the drug would be effective.

PB Enhances the Effect of Nerve Agent Antidotes

PB is an acetylcholinesterase inhibitor with a short half-life. It reversibly binds to peripheral acetylcholinesterase for several hours and temporarily blocks the irreversible inactivation of the enzyme by nerve agents. PB by itself does not counteract the effect of nerve agents, but it enhances the effects of antidotes and "is intended to be used in conjunction with protective garments, including a gas mask, and atropine and pralidoxime therapy at the first sign of nerve agent poisoning" (FDA 2003b, p 4).

The animal studies used to support the approval of PB demonstrated the differences among animal species. PB was effective as a pretreatment to soman exposure in rhesus monkeys. However, PB was *not* effective in rats, mice, or rabbits because they are naturally resistant to the nerve agent. These animals have high levels of carboxylesterase, which binds soman in the blood. Rats pretreated with a carboxylesterase inhibitor had a clear mortality support following PB plus atropine administration compared with untreated controls (FDA 2003b).

Cyanokit Dog and Human Data Indicates Effectiveness

The Cyanokit package insert indicates "that hydroxocobalamin is likely to produce clinical benefit in humans" (FDA 2009b, p 5). This conclusion was reached in studies done on dogs and in four French studies done on humans. Although clear conclusions could not be drawn from these four human studies, as they were not controlled and three were retrospective, in two of the studies there were

survivors after treatment, even though blood cyanide levels before therapy were generally considered to be in the lethal range (FDA 2006b).

The mechanism of action of the drug is the same in humans and dogs. Hydroxocobalamin binds with cyanide to form cyanocobalamin, which is a stable, nontoxic compound excreted in urine. Cyanide-poisoned adult dogs were assigned to hydroxocobalamin at 75 or 150 mg/kg or vehicle (0.9% saline). The primary endpoint was survival at 14 days (FDA 2006c, Table 1, page 11, and Table 4, page 29). Anesthetized dogs received IV administration of a lethal dose of potassium cyanide. Dogs then received hydroxocobalamin at 75 or 150 mg/kg or vehicle intravenously (IV) over 7.5 minutes[4]. The doses at 75 and 150 mg/kg "are approximately equivalent to 5 and 10 g of hydroxocobalamin (respectively) in humans based on both body weight and the C_{max} of hydroxocobalamin (total cobalamins-(III))…. Hydroxocobalamin reduced whole blood cyanide concentrations by approximately 50% by the end of the infusion compared with vehicle" (FDA 2006d, 2009b, p 5).

Two weeks after exposure and assigned intervention, 18% of the dogs in the placebo control group, 79% of dogs in the 75-mg/kg group and 100% of dogs in the 150-mg/kg hydroxocobalamin groups survived, respectively. "Histopathology revealed brain lesions that were consistent with cyanide-induced hypoxia. The incidence of brain lesions was markedly lower in hydroxocobalamin-treated animals compared to vehicle-treated groups" (FDA 2009b, p 5). Furthermore, this dog study directly contributed to determining an appropriate dose of Cyanokit in humans. These dose levels were found to correspond to a 5-g and 10-g dose, respectively, in a 70-kg human (FDA 2006b). The FDA reviewed the proposed animal efficacy study in the dog model via a special protocol assessment before initiation of the study.

In a clinical study that evaluated the effects of hydroxocobalamin administration to healthy subjects (no cyanide exposure), Cyanokit was shown to be well tolerated at the 5-g dose (FDA 2006c). The package insert indicates that "a second dose of 5 g may be administered by IV infusion", depending on the severity of the poisoning and the clinical response (FDA 2006e).

The FDA found that one well-controlled pivotal efficacy study in beagles was adequate for approval under the Animal Rule. Both groups that received Cyanokit had highly statistically significant improvement in survival compared with the control group at day 15; the mechanism of action appears to be the same in humans and dogs, and the animal study found a reduction in cyanide and an increase in cyanocobalamin in the Cyanokit-treated groups (FDA 2006f).

Newer Products under the Animal Rule

To date, no product developed using only animal efficacy studies has been licensed by the FDA. A prime example is the development of a therapeutic for inhalation anthrax. Human Genome Sciences (HGS) was awarded a contract from the Department of Health and Human Services (HHS) Biomedical Advanced Research and Development Authority (BARDA) of the Office of the Assistant Secretary for Preparedness and Response for developing and testing its human monoclonal antibody, raxibacumab, for inclusion in the U.S. Strategic National Stockpile. As a result of animal efficacy studies, in April 2009, HGS delivered 20,000 doses of raxibacumab to the stockpile to treat inhalational anthrax in an emergency. Three months later, "HGS received a second order for 45,000 doses to be delivered over a period of three years, beginning near the end of 2009. Both purchase awards were made under the Project BioShield Act of 2004 [P.L. 108-276], which is intended to hasten the development, purchase, and availability of medical countermeasures for the stockpile" (HGS 2011).

[4] The FDA pharmacology and toxicology review discusses the rationale for the route of challenge.

In 2009, HGS applied for the licensure of raxibacumab as treatment against inhalational anthrax pursuant to the Animal Rule (BLA 125349; FDA 2009c). Two doses of raxibacumab (20 and 40 mg/kg intravenously) were tested in randomized, placebo-controlled studies with rabbits and nonhuman primates and the higher dose was subjected to safety testing in human volunteers. The study concluded that a single dose of intravenous raxibacumab (40 mg/kg) improved the survival rate of monkeys (64%) and rabbits (44%) diagnosed with inhalational anthrax (Migone et al. 2009). However, an FDA advisory committee on October 27, 2009, suggested additional animal experiments and human safety studies,[5] which the company is currently undertaking (FDA 2009e; S. Bolmer, presentation to the Committee, see Appendix D).

REGULATION OF DRUG DEVELOPMENT

The FDA Center for Biologics Evaluation and Research (CBER) and the FDA Center for Drug Evaluation and Research (CDER) are responsible for regulating any products in the United States that would be approved under the Animal Rule. Current authority for the regulation of drugs resides in the Federal Food, Drug, and Cosmetic Act as amended by the Food and Drug Administration Modernization Act of 1997 (FFDC Act; 21 USC § 301 et seq.). Current authority for the regulation of biological products other than drugs (e.g., vaccines and monoclonal antibodies) is primarily Section 351 of the Public Health Service Act, 42 USC 201 et seq., and specific sections of the FFDC.[6]

Drugs and biological products have the same general development pathway. Both drugs and biological products are subject to the IND application regulations (21 CFR Parts 312, 314 and 316). "A sponsor who wishes to begin human clinical trials…must submit an IND" (FDA 2009d) to the appropriate FDA division. "The IND describes the [product,] manufacturing, and quality control tests for product release. The IND also includes information about the product's safety testing" (FDA 2009d) and pharmacokinetic testing in animals. In the case of vaccines, the immunogenicity testing in animals would be provided. In addition, the IND would include the proposed clinical protocol for a study in humans.

In the human studies, premarketing clinical trials for new products are usually done in three phases. In phase 1, safety and pharmacokinetic (or, in the case of vaccines, immunogenicity) studies are "performed in a small number of closely monitored subjects. Phase 2 studies are dose-ranging studies

[5] Additional animal studies were suggested in response to (1) whether the evidence from the animals treated with the 40 mg/kg dose predicted the response in humans with inhalational anthrax (i.e., accurate depiction of the course of disease in humans; timing of treatment after exposure; benefit of raxibacumab in relation to timing post exposure; additional studies with special populations, including children); (2) whether the evidence supports the conclusion that raxibacumab will not diminish the anticipated efficacy of antimicrobials against inhalational anthrax (i.e., timing of administration; proper dosing of antimicrobials; use of different antimicrobials); (3) whether evidence should be requested that raxibacumab make a contribution to the efficacy over the antimicrobial alone in rabbit and nonhuman primate models (i.e., standard or suboptimal doses of antibiotics in humans to see a contributing effect of raxibacumab; use of timing to mimic the course of infection in humans; use of rabbits instead of primates; when to start treatment; the state of the animal prior to the treatment with raxibacumab); (4) whether to further evaluate central nervous system (CNS) effects of raxibacumab (i.e., in rabbits to determine if the antigen-antibody complex is the reason for increased pathology due to complement activation). Additional studies to evaluate safety in humans were suggested including studies in pediatric and elderly populations as well as studies to distinguish the effects of the infection, toxins, and the immune response. These studies should address the issue of timing (FDA 2009e).

[6] For definitions of biologics, see "What Are 'Biologics' Questions and Answers" at http://www.fda.gov/AboutFDA/CentersOffices/CBER/ucm133077.htm.

and may enroll hundreds of subjects. Finally, phase 3 trials typically enroll thousands of subjects and provide the critical evaluation" (FDA 2009d) of efficacy as well as important additional safety data required to make a risk-benefit assessment of the product. In certain novel cases, the FDA may seek input from the appropriate FDA advisory committee, especially for issues relating to trial design and endpoints, before the initiation of phase 3 studies. The FDA advisory committee members are external experts, typically physicians, scientists, statisticians, and a consumer representative.

Following the completion of all three phases of clinical development, the sponsor can make a determination on whether the data support the submission of a marketing application to the FDA. The marketing application would be either a new drug application (NDA) or a biologics license application (BLA), depending on the type of product.[7] The NDA or BLA must "provide the multidisciplinary FDA reviewer team with adequate efficacy and safety information" to make both a risk-benefit determination and a recommendation to approve or not approve the product. During this stage, the manufacturing facility undergoes a preapproval inspection for new products (FDA 2009d).

The FDA may convene an advisory committee meeting before taking a final action on a BLA or NDA. In general, products (or new indications) that are novel or present new issues would be evaluated by the appropriate FDA advisory committee. The committee provides advice to the FDA regarding the safety and efficacy of the product for the proposed indication as well as what postmarketing clinical trials should be considered, if applicable.

As part of the approval process, the FDA works with the applicant on the product package insert and other labeling.

Following approval, the FDA continues to oversee the manufacture of products and their safety. Specifically, the FDA performs periodic facility inspections on an ongoing basis and it also engages in post market surveillance, i.e., the process intended to identify safety issues or new problems prior to approval, and any problems that occur because a product may not be used as described in the product package insert. Medwatch is the FDA Safety Information and Adverse Event Reporting Program, "…a computerized database designed to support the FDA Postmarketing Safety Surveillance Program for approved drug and therapeutic biologic products" (FDA 2009d). For vaccines, postmarketing surveillance is accomplished separately with the Vaccine Adverse Event Reporting System (VAERS), cosponsored by the FDA and the Centers for Disease Control and Prevention (CDC).

Specific studies are often conducted after a product has been approved to obtain further information about a product's safety and efficacy. "Postmarketing *requirements* (PMRs) include studies and clinical trials that sponsors are *required* to conduct by statute or regulation. In contrast, postmarketing *commitments* (PMCs) are studies or clinical trials that a sponsor has agreed to conduct" (FDA 2009d); however, the studies or clinical trials are not required by statute or regulation. PMRs and PMCs are listed in the FDA approval letters. Results of these postmarketing studies are often used to modify product labeling.

[7] If the application is for a new indication for an already approved product, the submission would be an NDA or BLA efficacy supplement.

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4

Developing New Animal Models for Biodefense Research

This chapter addresses the process and feasibility of developing new animal models for biodefense research. The previous chapter established that the ability of animal models to predict the human condition is not absolute and that collection of human clinical data is critical. **The Committee on Animal Models for Assessing Countermeasures to Bioterrorism Agents concludes that focusing on the creation of new animal models—that is, continuing to rely exclusively on the use of animals for efficacy studies—is not warranted at this time**. Although new models, such as hamsters, New World nonhuman primates, pigs, or bats, may be useful for basic research purposes, they will eventually encounter the same problems seen in the better-defined animal models currently in use.[1] Instead, the Committee suggests that it is more useful to use different approaches that "support the qualification[2] of animal models" (DHHS 2010, p 11) and increase understanding of how animal data may more consistently predict the human response as follows:

- Improve the quality and quantity of collected data about the natural history of the diseases studied and data from human patients in clinical settings.
- Continue and improve the acquisition of expanded data from phase 1 human safety trials.
- Maintain frequent interactions with the FDA.
- Focus on the reproducibility and scientific relevance of the compartmentalized[3] animal model rather than on its validation.
- Develop strategies for interspecies comparisons, such as compartmentalization.

[1] As discussed in Chapter 2, current animal models do not adequately reflect the human condition; their validity cannot be easily evaluated due to paucity of human data; results from different species may not be comparable partly due to biocontainment constraints and partly due to methodological differences; and they are complex and expensive to develop.

[2] The Animal Rule does not discuss the validation of animal models (FDA 2002).

[3] Compartmentalization means to plan experiments that will yield information from components of the animal (organs, cells and systems) rather than data derived from the whole organism (for additional discussion see page 58).

USE OF HUMAN DATA TO IMPROVE THE VALUE OF ANIMAL MODELS

Postmarketing Clinical Studies

Before FDA approval, drugs and biological products must be tested in three phases of clinical trials to determine efficacy in humans. Increasing numbers of human volunteers will be used in the three phases to determine safety, and when feasible and ethical, under conditions that reflect the natural exposure or disease condition. Once the drug or biological product is approved, the FDA can require additional postmarketing clinical studies to determine whether there are safety issues that are revealed in larger populations than were tested in premarketing studies to ensure the quality and consistency of manufacturing and to gather more data on efficacy when the drug is used under normal clinical conditions. These postmarketing clinical studies are required for products that are approved under accelerated approval provisions. These provisions are for serious or life-threatening conditions; for marketed drugs approved in adults which have the potential for benefit in children; or more recently, for products that have been approved under the Animal Rule (21 CFR Parts 314 and 601 [2002]). In these cases, the FDA requires postmarketing commitments that outline the clinical studies that will be undertaken and the time frame under which they will be carried out. In addition, the agency can require postmarketing studies to determine whether there are reasons to withdraw the approval of the product; i.e., whether there is a known or potential serious risk related to the use of the drug. The FDA requires annual reporting of the status of the postmarketing studies until it determines that the commitments have been fulfilled (21 CFR Part 314.81(b)(2)(vii) [2011]).

For drugs and biologics approved under the Animal Rule, the FDA requires postmarketing studies or clinical trials to "verify and describe the drug's [or biological product's] clinical benefit and to assess its safety when used as indicated when these studies are feasible and ethical" (FDA 2002, p 37995, 37997). The FDA requires that applicants submit a plan or an approach, including the appropriate due diligence to identify opportunities to conduct these studies, and to carry out the clinical studies if and when it becomes feasible and ethical to do so. However, due to the nature of these products and the pathogens they are meant to counter, opportunities to conduct these clinical studies are rare and may only be possible under exposure to a chemical, biological, or nuclear threat. For some products, however, particularly for those used in the prevention or treatment of (emerging) infectious diseases, there may be particular environmental or natural conditions that provide an opportunity to collect human clinical efficacy data under natural exposure. In fact, the Animal Rule treats the use of a product approved under the rule in response to an exposure as a clinical trial from which essential data can be obtained (Walker and King 2011).

These clinical studies also offer the opportunity to evaluate the relevance and predictability of the animal models that were used in the approval process. These studies, therefore, represent important opportunities to refine the animal model systems, to evaluate the biomarkers identified and measured, and to correlate the clinical findings in humans to those seen in the animal models. By identifying and collecting data using the appropriate biomarkers[4] and correlating clinical information to existing animal data, it should be possible to improve understanding of the relevance of the animal model to human clinical responses. Such data would be particularly informative to models developed with a product-neutral approach, which, by exhibiting a broader application profile, may be useful in "unknown-unknown" exigencies (see Chapter 2, p 30). Therefore, it would be useful for postmarketing

[4] Biomarker is a biological characteristic that can be objectively measured and evaluated as an indicator of normal, pathogenic, or pharmacological responses or as the target of a therapeutic intervention. Biomarkers provide insight into disease progression, prognosis, and response to therapy.

commitments to include plans that provide data to evaluate the relevance of the animal model to the human condition.

Natural Disease Outbreaks

Many of the infectious disease threat agents that are the target of products developed under the Animal Rule occur naturally in many places of the world and may be responsible for outbreaks of disease in humans (Warfield et al. 2006). These disease outbreaks represent potential opportunities to collect prophylactic and therapeutic clinical data on the efficacy of drugs and biologics approved under the Animal Rule and to collect data to determine the relevance of the animal models used. Therefore, it is important to identify such potential opportunities and collaborations early in the clinical development process. For the countries where outbreaks are known to occur, the products approved under the Animal Rule also represent potentially important tools for public health officials to reduce morbidity and mortality in exposed populations, to protect and treat first responders during outbreaks, and to support ongoing surveillance and containment efforts.

For drugs and vaccines where natural and environmental exposure can occur, postmarketing commitments can be developed that include (1) the identification of potential sources of outbreaks in humans; (2) the opportunities for supporting first responders and surveillance and containment efforts; and (3) the development of a strategy to engage key partners from endemic country academic institutions, public health, and governmental agencies at an early stage of clinical development or at the time of approval.

Phase 1 Human Safety Trials

Safety evaluation and verification of clinical benefit of products approved under the Animal Rule is required and studied in human volunteers under the conditions described under 21 CFR Parts 312 and 320 [2010]. As mentioned in Chapter 2, about 20% of drugs fail due to safety concerns, many of which were either not observed or not predicted by animal trials. Even though safety trials are not a substitute for clinical trials with humans, the combined purpose of safety trials under the Animal Rule indicates that useful data can be collected to inform the efficacy of the product. For instance, if pharmacological responses were studied and biomarkers were developed in the animal models, biomarker levels in humans could be monitored and later correlated to the animal-based data.

Expanded data acquisition from these trials could be useful in multiple ways, especially if the duration of the trials were expanded and the trials structured to (1) mirror the anticipated treatment regimen in humans, and (2) reflect the heterogeneity of the general population.[5] Furthermore, surrogate markers for efficacy can be tested in safety trials, such as finding the level and functionality of antibodies induced,[6] the blood or tissue concentration of drugs relative to the critical bactericidal and antiviral concentrations, and the actual modification of immune responses.

[5] The FDA advisory committee tasked with the evaluation of the application of Human Genome Sciences for licensure of raxibacumab under the Animal Rule (see Chapter 3 for more details) requested that additional safety studies in humans include pediatric and elderly populations (FDA 2009b).

[6] Data from human safety studies of raxibacumab show that blood concentrations of the antibody can be used as a surrogate endpoint predictive of clinical benefit (Migone et al. 2009).

INTERACTIONS WITH THE FOOD AND DRUG ADMINISTRATION

The Animal Rule regulatory pathway is relatively new to the FDA and the research community. As discussed in Chapter 3 only two drugs have been approved under this rule since 2002; both of them were repurposed for use as medical countermeasures. Most of the proposed approaches in this report are unlikely to rely on a precedent example from a marketed product. Thus, early interactions with the FDA are critical for products that will be considered under the Animal Rule regulatory pathway, as presented in Box 4-1.

BOX 4-1
Formal Meetings with the FDA

Pre-investigational New Drug (IND) Application Meetings

Prior to the submission of an initial IND, the sponsor (applicant) can request a meeting with the FDA to review and reach agreement on the format of the IND (21 CFR § 312.82 [1998]), the phase 1 clinical protocol, the design of animal studies needed to support human clinical testing (e.g., protocols for toxicological studies), and chemistry, manufacturing, and controls (CMC) information. The CMC information includes a description of the product potency assay and early product stability assessment protocols. An important goal of a pre-IND meeting (regardless of the regulatory pathway) is to identify issues that may lead to a delay in the initiation of the phase 1 clinical trial, i.e., the "clinical hold" (21 CFR § 312.42 [2009]; FDA 1995, 2001). The overall plan for investigating the product should be considered in the context of the proposed clinical indications, including a proposed "indications and usage" section analogous to that seen on the package insert. Issues regarding the potential for fast-track designation may be discussed.

Early discussions regarding Animal Rule specific issues may also occur at the pre-IND meeting or separately. Examples of pertinent issues include the appropriateness of using the Animal Rule regulatory pathway for the specific clinical indications; pilot animal efficacy studies (protocols or data); and iterative determinations for the pivotal animal studies, such as the relevant dose level based on prior pilot animal and human studies.

End-of-Phase 1 (EOP 1) Meeting

A sponsor may request an EOP 1 meeting for a product with fast-track designation after completion of early phase 1 clinical studies to review the phase 1 data and reach agreement on clinical plans for the phase 2 program (21 CFR § 312.82(b) [1998]). Emerging data from pilot animal pharmacokinetic and efficacy studies, or animal study protocols, can also be discussed. For drugs for life-threatening diseases, the FDA will provide its best judgment, including whether pediatric studies will be required and, if so, whether their submission can be deferred until the product has been approved" for adults (21 CFR § 312.82(b) [2010]). Other issues can also be covered, e.g., emerging CMC and assay validation questions.

End-of-Phase 2 (EOP 2) and Prephase 3 Meeting

The purpose of this meeting is to review the phase 2 clinical data before proceeding to phase 3, to evaluate plans for the phase 3 clinical program and clinical protocols, and to identify any additional information necessary to support a marketing new drug application (NDA) or a biological license application (BLA) for the proposed clinical indications, e.g., unresolved CMC issues (21 CFR § 312.47(b)(1) [2002]). For products developed under the Animal Rule regulatory pathway, the phase 3 clinical studies may include the definitive clinical pharmacokinetic study (or immunogenicity study for vaccines) as well as larger well-controlled safety studies. The phase 3 equivalents for animal studies include animal pharmacokinetic and animal efficacy studies.

Specific Information on Pivotal Animal Studies (Phase 3)

When devising a development plan and protocols for animal studies, the applicant should recognize that certain principles of clinical trial design will also apply to the animal studies; therefore, multidisciplinary collaboration among research scientists, clinical trial experts and biostatisticians is highly recommended. The principles for adequate and well-controlled human efficacy trials should be applied (as appropriate) to the animal studies (described in 21 CFR § 314.126 [2010]). The animal study protocols and statistical analysis plan for the animal efficacy studies (including control groups, justification, and clear prospective

primary and secondary endpoints) are provided to the agency with sufficient time for FDA review and comment before conducting the study. The analysis of the primary efficacy endpoint will form the basis for approval.

Other studies in support of an NDA or BLA, such as the clinical or animal pharmacokinetic interaction studies (e.g., between the investigational drug and an approved drug that would be used concurrently), may be necessary.

"VALIDATION" OF ANIMAL MODELS FOR BIODEFENSE RESEARCH

A question that arises when applying the Animal Rule is whether or not an animal model is validated. Validation is a regulatory concept that describes the suitability of an analytical procedure for its intended purpose and includes such qualities as accuracy, precision, repeatability, intermediate precision, specificity, detection limit, quantitation limit, linearity, and range (ICH 2005).

Such attributes do not apply to animal models and the Animal Rule does not mention "validated" animal studies. However, reproducibility and scientific relevance are essential qualities of successful animal models because they can lead to standardization of the model.[7] The field of regulatory assessment (which includes drugs, chemicals, vaccines, cosmetics and pesticides), which depends both on animal testing and alternative methods to animal experiments, relies on a concept of validation that incorporates, among other factors, both reproducibility and scientific relevance, as depicted in Figure 4-1.

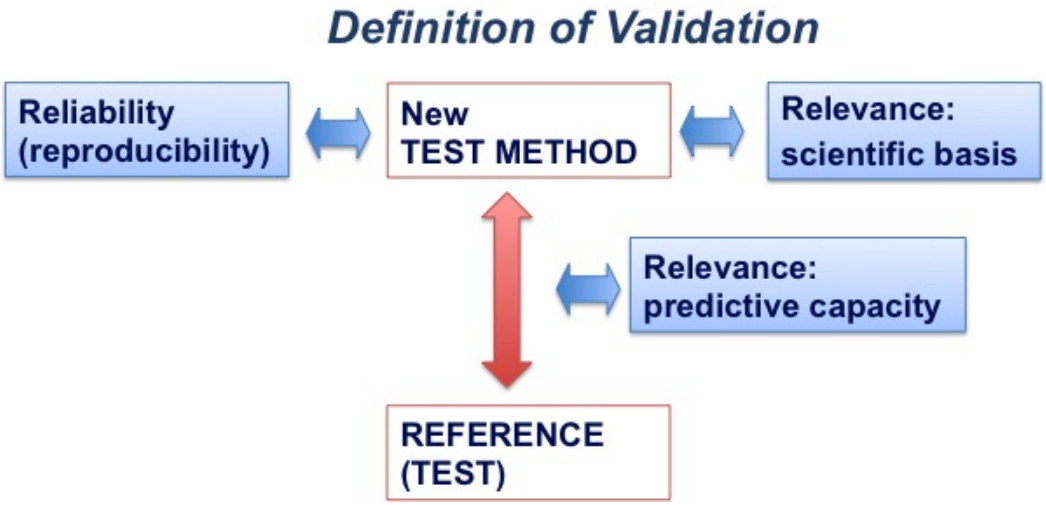

FIGURE 4-1 Definition of validation: "Validation is a process in which the scientific basis and reproducibility of a test system, and the predictive capacity of an associated prediction model, undergo independent assessment." SOURCE: Adapted from Hartung 2007.

In this diagram the new test method (i.e., the animal model) is compared to the reference test. If no such reference test exists then a consensus standard, agreed upon by experts, is utilized, e.g., a number of positive and negative substances (Hoffmann et al. 2008). The results of the new method are

[7] The development of such a model by NIAID for inhalational anthrax is discussed in Appendix C.

then compared with the reference results. Three principal aspects of validity of the new method are examined: (1) reliability (i.e., reproducibility), (2) relevance of the model (i.e., its scientific basis), and (3) predictive capacity of the new method (i.e., the animal model) for the reference results. In addition, the new test is subjected to quality control (i.e., standardization).

In the case of animal models for countermeasures research there usually are no reference data available—that is, human pathological findings and clinical data are insufficient, there are no failed or successful drugs, and no reference test model has been established—with which to compare outcomes from the animal studies. Thus, a traditional validation assessment would not be possible.

Evaluation of the validity of a model is "an evolving process that is never completed because the models are always subject to further definitive reexaminations and revalidation as new technology becomes available" (Anderson and Tucker 2006). However, a model's qualification (DHHS 2010) may be assessed by an approach that utilizes only some of the elements presented in Figure 4-1; the model's reproducibility is coupled with an assessment of the scientific relevance of the model to the human condition that will include a robust analysis of modes of action in response to pathogens. Comparative analyses of outcomes from different animal species (see next section) may be an equally important source of information.

Taking into account the limitations of animal models outlined in Chapter 2 and the dearth of human reference data, the model's reproducible response to a pathogen challenge across different laboratory settings and methods will help characterize or qualify the model rather than seek to validate it.[8,9]

COMPARATIVE BIOLOGY AND COMPARTMENTALIZATION IN ANIMAL MODEL DEVELOPMENT

An important problem facing animal modelers is that there is no roadmap for what makes an animal model good or bad. Historically, models were often deemed inferior because one protein in a critical pathway in one animal species was not identical to the corresponding pathway or protein seen in humans. A good example is found in the history of nitric oxide (NO), one of the primary effector molecules against a variety of intracellular pathogens (Chakravortty and Hensel 2003). Although murine macrophages readily produce NO, human cells often cannot (Yang et al. 2009). Consequently, the utility of murine models to investigate the role of NO was not obvious. However, those models could be quite useful in investigating most of the other aspects of host–intracellular pathogen interactions with the possible exception of the role of NO in humans. Thus, it would be more useful to compare the systems and pathways that lead up to a host response within a species, across species, and with humans rather than focusing on a single gene or protein or particular genes or proteins. This strategy is known as compartmentalization. If this strategy were applied to NO research, it would show that the mouse and human share most of the major pathways leading up to the production of interferon γ and subsequent macrophage activation except for the production of the final effector NO.

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[8] A different set of criteria (Technology Readiness Levels) for medical countermeasures and detailed description of the development stages of animal models to support approval of countermeasures under the Animal Rule have been developed by the working groups of the Public Health Emergency Medical Countermeasures Enterprise for use across the public and private sector; https://www.medicalcountermeasures.gov/TRL_Page.aspx.

[9] In its *Draft Guidance to Industry* the FDA has provided a list of the "essential data elements of an animal model." As the document clearly states, however, "These elements serve as a guide. They may be modified or revised…" (FDA 2009, p 16-17).

The basic premise of compartmentalization is that each species is made up of a variety of physiological compartments that contribute to the host response to an infectious agent: One compartment is the innate immune response, a second compartment is the acquired immune response, a third includes the determinants of the pathogen's tropism for that species, and so on. Pathophysiological data from humans can be used to identify the specific compartments of the animal models that are most relevant to the human condition and that can be the focus of research protocols and data evaluation. The possibility that these compartments overlap among several animal models and are not mutually exclusive would suggest a consistent response among different species. This response can be extrapolated more confidently to the human condition. Some of these overlapping compartmental responses may even occur in species in which important deviations from the human responses have been observed.

It is necessary to develop compartmentalization strategies and algorithms for comparing infectious disease models across several species to increase the likelihood of extrapolating the product effect in humans. This type of strategy may relieve the pressure to find one or two optimal models that can be used to address all aspects of the extrapolation and to focus all efforts and resources.[10] For example, because susceptibility to anthrax is variable among animal species based on the role of toxin or capsule (see Chapter 2, pages 29-30), a synergistic strategy has been proposed to increase the relevance of anthrax animal models in the holistic modeling of the human disease (Goossens 2009). In variola studies, which are constrained by the species-specific tropism of individual poxviruses (McFadden 2005), data from multiple species that respond to different poxviruses (cowpoxvirus in murine species and monkeypoxvirus in nonhuman primates) can be combined. Otherwise, a suboptimal nonhuman primate model of variola that is challenged with massive nonphysiological doses of the virus must be relied upon (Jahrling et al. 2004).

Another advantage of the compartmentalization approach is the potential repurposing of animal models to discreet components of countermeasure development for which they may be better suited. For example, the results from the use of antitoxin therapeutics translate well from the murine model infected with the anthrax Sterne strain (Chapter 2, page 29) to the rabbit model of inhalational anthrax (Loving et al. 2009). This result suggests that, in some cases, the model selection for the development of countermeasures could be guided by the specific experimental question or the target. In other words, in the compartmentalization approach, the animal model is specialized (i.e., made to fit) to only one pathophysiology component, the toxin, so that collected information is specific to the host–toxin interaction and not to the whole organism and host–pathogen interaction.

OPTIMIZING CURRENT ANIMAL MODELS

The production of relevant and effective medical countermeasures to biothreats would greatly improve if the focus were shifted from developing new animal models to improving the extrapolation of animal data from the current models to the human response. This shift can be accomplished by cultivating alternative or underutilized sources of information, such as the data collected from current models and human populations.

[10] An example of the difficulty of developing optimal animal models in different species is presented in Appendix C in the section *4.1 Anthrax*.

The Committee recommends that the TMT adopt the following strategies to improve the usefulness of current animal models:

- **To control interspecies variability and improve the comparativeness of infectious disease models across different species, adopt the concept of compartmentalization. As each species is made up of a variety of physiological compartments that contribute to the host response to an infectious agent, compartmentalization is a strategy to compare the systems and pathways that lead up to the host response within a species, across species, and with humans rather than focusing on a single gene or protein or particular genes or proteins.**
- **To support the qualification of animal models as an alternative to validation, establish the compartmentalized model's scientific relevance and reproducibility across different methods and laboratories. These comparative datasets may subsequently be used to define appropriate criteria to characterize or qualify vs. validate the animal model.**

The Committee also recommends the following to improve the usefulness of the information derived from human populations:

- **To address the dearth of data from human populations, expand the collection of data from patients in outbreak zones and from postmarketing studies. In addition, expand the acquisition of data from phase 1 safety trials by (1) increasing the duration of these trials; (2) diversifying the enrolled subjects to mirror the general population; and (3) including the anticipated treatment in the field as part of the trial protocol.**

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5

Alternative Approaches to Animal Testing for Biodefense Countermeasures

This chapter examines alternative approaches to the use of animals in the development of medical countermeasures against biothreats. It presents the concept of the Three Rs as a basis to safeguard good science while improving laboratory animal welfare. It briefly describes a number of in vitro and in vivo methods that support the Three Rs and humane endpoints. **The Committee on Animal Models for Assessing Countermeasures to Bioterrorism Agents concludes that absolute replacement of animal models in countermeasure development is not possible at this time and that in vitro and in silico methods are not advanced enough yet (in part due to absence of human data) to reliably replace animals in biodefense research**. Recognizing that the premise of the Animal Rule is the use of animals and that the Animal Welfare Act (AWA; 7 USC § 2131-2159) requires the consideration of alternatives, the Committee recommends to embrace and further develop alternative options to (1) take advantage of new (clinical and epidemiological) data; (2) correlate those new findings with outcomes from established animal models; (3) improve the welfare of animals used in countermeasure development and testing; and (4) strive, where possible, to replace nonhuman primates as the animal of choice in biodefense research. The Committee reiterates the fundamental need for data from human populations (versus laboratory animal species) as the crucial driver for the development of in vitro and in silico pathways. **Further, the Committee concludes that changing the standard practice of animal experimentation to approximate the clinical course of treatment that humans may receive could provide a more reasonable expectation of the usefulness of certain countermeasures during development.**

GENERAL PRINCIPLES OF ALTERNATIVE APPROACHES

In 1959, Russell and Burch formulated three principles to reduce the numbers of animals used in experimentation. These are known as the Three Rs: refinement, reduction, and replacement (absolute or relative).[1] The validation process of regulatory testing has over the years incorporated many methods that support one or more of these principles for two main reasons: (1) only regulated and standardized animal tests that are repeatedly carried out over a long period of time (typical timeframe 12 years) and tests that have enormous costs warrant a formal validation process to be accepted as such; and (2) in the area of safety assessments, it is especially difficult to abandon an established test because safety standards could be lowered. In other areas of research and development, especially in the agent discovery phase, replacement of older with more advanced methods is more common due to constant pressure for more predictive and less costly tests.

In the case of developing countermeasures for bioterrorism agents where absolute dependence on animal models for efficacy testing serves to replace human clinical trials, the Three Rs provide a good framework to reduce animal use and minimize the animals' pain and distress.[2] Because adoption of alternatives was driven by both animal welfare considerations and scientific advances in our understanding of biological phenomena, their utilization is often in the best interest of the study. Although alternatives do not compensate for the lack of clinical efficacy trials with human participants, they can enable technologies from which more information is gained than that gained by animal tests alone. In addition, they reduce the use of precious and expensive resources and reduce animal pain and distress, often resulting in improved quality of research outcomes (NCR 2008, 2009, 2011; Wolfer et al. 2004).

Applying the Principle of Refinement

The best possible treatment of animals starts with attention to husbandry (AWA; 7 USC § 2131-2159). Social housing and enriched environment within the cages, especially for the highly social nonhuman primates often used in these studies, while taking into consideration the scientific needs of the study, represent key strategies to avoid or reduce distress[3] (NRC 2008). The use of analgesics and anesthetics is mandatory, not only for the alleviation of pain and distress, but also because it represents a more realistic approximation to the treatment of human patients.[4,5]

The development and use of protocol-appropriate humane endpoints, especially the early termination of studies at time points that indicate that animals are unlikely to recover, minimizes pain

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[1] The utility and applicability of the Three Rs has been described in various documents. For additional information, see the *Guide for the Care and Use of Laboratory Animals, Eighth Edition* and references therein (NRC 2011), and the website for the National Centre for the Replacement, Refinement and Reduction of Animals in Research (http://www.nc3rs.org.uk/).

[2] The usefulness of the Three Rs and the employment of alternatives in regulatory safety testing are not discussed here as safety testing does not fall under the auspices of the Animal Rule.

[3] The ability to provide enriched environment or social housing in biocontainment facilities may be difficult, extremely limited, or impossible. Although it is a necessary husbandry and animal welfare provision, methods to enrich the environment or house social animals in a biocontainment facility have not been studied.

[4] It should be noted that the quality of care for human patients is not universally identical and that analgesics and anesthetics may not be available in natural outbreak settings. Therefore, different animal research protocols may be needed for the development of treatments under these conditions.

[5] Non-administration of analgesics should be scientifically justifiable and accepted by an Institutional Animal Care and Use Committee (IACUC; Animal Welfare Act; 7 USC § 2131-2159) and employed as rarely as possible.

and distress without compromising the result of the study[6] (Chapter 5 of *Recognition and Alleviation of Pain in Laboratory Animals (*NRC 2008); Nemzek et al. 2004; NRC 2011; Olfert and Godson 2000). Conversely, insistence on death or even moribundity as an endpoint is questionable, as signs of irreversible decline are well established for all common laboratory animal species.[7] It is important that early termination studies include complete necropsies and histopathological examination accompanied by appropriate agent isolation from tissues to determine if the killed animal was, in fact, unlikely to survive. Further, early endpoints ought to be verified before embarking on larger studies to ensure that studies are not needlessly repeated. At a minimum, if natural history or descriptive studies require an understanding of events proximate to death or a time-to-death estimate, then these data should be used where possible as a historical benchmark to estimate fatal outcome without needing to actually follow a full disease course in a moribund animal.

Looking at the Numbers: Reduction

Most measures to reduce the number of animals used are often justified in terms of avoiding pain or distress and of saving resources, especially in studies conducted in biocontainment facilities. Well-chosen statistical methods, such as appropriate power calculations of group numbers, should always be part of the experimental design, and knowledge of historical data about the variance of the anticipated results can help to select the appropriate sample size. Tiered testing strategies (e.g., treating individual animals or small groups sequentially and not in parallel) and use of qualified pilot studies allow for studies to be terminated early if no effect is observed in the first few animals.[8] As discussed in the previous section, it may be possible to avoid the use of untreated contemporary control groups if historical data can be employed. Similarly, sharing control groups among multiple experiments performed at the same time may also lower the number of animals required. Careful selection of dosages and omission of unrealistic treatment groups further reduces animal use.

The broader use of inbred murids has helped to reduce the variability of experimental results and thus the size and number of groups required. Consequently, inbred animals are often selected for this reason, although their use does not reflect the variability of the human population (also stated in Chapter 2, Table 2-9; for an approach that embraces genetically diverse murine models, see page 69). The use of early, informative endpoints derived from complementary in vitro methods (e.g., estimation of effective or maximal tolerated doses by effective concentrations in vitro and in vitro metabolism studies with hepatocytes or microsomes to exclude the use of species that do not reflect human metabolism) can further reduce animal numbers and refine the experiments by minimizing pain and distress. Other noninvasive methods, such as imaging technologies and telemetry, that allow

[6] Biomarkers and signatures of toxicity that are often derived from nonanimal models are useful tools that make animal studies more sensitive or facilitate earlier termination (with humane endpoints) without loss of information.

[7] The development of (early termination) endpoints and guidelines for when animals should be euthanized is a highly desirable animal welfare practice, and as such discussed in laboratory animal care and use regulations. Under the AWA, such provisions are part of the research protocol and subject to IACUC approval. The Committee recognizes that the above can be at odds with the 3rd criterion of the Animal Rule that "the animal study endpoint is clearly related to the desired benefit in humans, which is generally the enhancement of survival or prevention of major morbidity" (FDA 2002, p 37989). That is why pilot studies and determination of biomarkers or endpoints through small sample sizes are important. Further, careful and focused clinical observation of animals can identify entrance into an irreversible state of decline followed by immediate euthanasia.

[8] Such approaches may require larger group sizes for statistical power.

monitoring of the same animal over the entire course of an experiment, instead of sacrificing more animals at different time-points, also reduce group-sizes while preserving statistical power.[9]

Focusing on Replacement

While opportunities for in vitro replacements are available to a greater extent in toxicology and safety assessments (e.g., NICEATM and ICCVAM test method evaluations; http://iccvam.niehs.nih.gov/methods/methods.htm), non-sentient test systems in infectious disease research generally and in studies under the Animal Rule particularly are limited. Some opportunities lie in ex vivo approaches where animals or human volunteers are treated with the product in development and only tissues or blood are subsequently exposed to pathogens or used for measurements. Tissue engineering methods, including artificial organs and organotypic cultures, can sometimes reproduce the physiological environment to study aspects of the course of infection, but extrapolation to the in vivo conditions and the systemic multifactorial components of host defense are limited. For further exploration of this topic as it pertains to studies under the Animal Rule, see section *In vitro tools and replacement strategies* (below).

ANIMAL EFFICACY STUDIES ARE CLINICAL TRIALS

Clinical trial designs for efficacy mandate that human subjects must be protected from undue risk when participating in clinical research activities (45 CFR 46 [2009]; National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research 1979). This protection includes the provision of clinical standard of care in addition to the product being evaluated (unless the standard of care is contraindicated). However, efficacy testing of a new drug or vaccine in animals routinely involves the administration of only the test product. Although this has been the standard practice in animal research protocols, such reliance solely on the test article to demonstrate efficacy may produce a misleading model of what the human counterpart may actually experience and lead to false or incomplete data on the effectiveness of the test product. Patients with dyspnea and acid and base imbalances due to pulmonary insufficiency, for example, would have to be provided with oxygen and intravenous electrolytes while enrolled in a trial to test the efficacy of a novel bronchodilator. Similarly, patients with congestive heart failure participating in an efficacy trial for a drug intended to increase the strength of myocardial contractions, would concomitantly be given diuretics (presuming sufficient kidney function) to minimize pulmonary congestion. In either example, although the test product could be efficacious for neutralizing or reversing the initial insult, the patient could still succumb from underlying or preexisting complications that were not treated by the test product (Miller and Silverman 2004).

The same clinical standard of supportive care certainly applies to persons exposed to a bioterrorism agent, regardless of whether they are lightly exposed and asymptomatic or suffering from organ failure. Severe dehydration and hypotension resulting from a highly infectious pathogen (e.g., acute diarrhea, septic shock, and hemorrhagic fever) would be treated with blood volume and blood pressure restoratives even though the origin of disease was microbial and the test product being evaluated was an antibiotic or antiviral drug.

The incorporation of supportive veterinary care in animal efficacy testing of countermeasures against biothreats is recognized by regulators as both reasonable and informative. In the 2009 *Draft*

[9] The use for in vivo imaging strategies in product development for biothreats remains largely unrealized. For these techniques to be used in the preclinical arena, validation of imaging as a correlate of bacterial or viral burden is necessary.

Guidance for Industry concept paper, the FDA described important components to be included in preclinical protocols for demonstrating efficacy under the Animal Rule. In that document, it is advised that "studies should be designed to mimic the clinical scenario and achieve meaningful outcomes comparable to the endpoints desired in humans. In some instances, supportive care should be administered to the animals as part of the study design" (FDA 2009a; see Appendix B). Not providing similar medical interventions to an animal subject when assessing preclinical efficacy may result in false (negative) conclusions about corresponding efficacy in humans. A test product could be sufficiently effective when combined with reasonable supportive care, but it could "fail" if evaluated alone. The acute need for additional biodefense medical countermeasures is not served when candidate drugs and vaccines are abandoned that may, indeed, prove efficacious when tested in a comprehensive medical fashion.

Employing an expanded spectrum of clinical care rather than relying on a single test product for efficacy testing has other advantages beyond not prematurely discarding promising drugs and vaccines. By more closely mimicking the broader scope of clinical care provided to patients, one may identify which specific ancillary care regimens, if any, contribute most to the efficacy of the test product. This information might lead to a critical component for the final label of the approved drug. In addition, longer survival of animal subjects due to an expanded repertoire of clinical support could result in better predictive models. If animals die too quickly,[10] the pharmacokinetics and drug metabolism of the test product or the absence of effects of increased time on an effective immune response may not replicate or approximate the expected timelines in patients, resulting in misleading findings.[11] Furthermore, subtle yet important differences in test products or dosages may become evident over a longer time frame of therapy due to prolonged animal survival. Objections to including an expanded array of clinical care for animal subjects in efficacy testing protocols usually involve one of two themes. First, anything administered to the animal besides the test product could interfere with the "true" efficacy properties of the product in question, possibly leading to (false) positive conclusions when the test product is not otherwise strong enough as a therapeutic or preventative agent. A counter-argument to that objection is that efficacy should address only the specific effects of that pathogen or toxin; if nonspecific complications represent the actual disease, then one should focus on efficacy testing specific to those sequelae.[12]

The second objection is that supportive-care components are not compatible with 21 CFR Part 58 (Good Laboratory Practice for Nonclinical Laboratory Studies; GLP) because the components may introduce high levels of variation that cannot withstand a quality-assurance audit of that study, or they may create many expensive complexities. If, however, quantitative thresholds are established for anticipated clinical signs (e.g., fever) and standardized supportive interventions in advance, such an

[10] Lethality in animals may be due to secondary causes, such as severe dehydration or hypothermia as a consequence of being too sick to eat, drink, or move around rather than specific or primary effects of the disease or the product tested.

[11] Metabolism and thus pharmacokinetics can differ between humans and animals (Martignoni et al. 2006). The difference is relevant not only for extrapolations to human kinetics but also for its impact on drug efficacy studies, i.e., where, when, and for how long are effective tissue concentrations reached. Furthermore, drug pharmacokinetics may change under disease conditions and may be affected by the severity of disease.

[12] As discussed in chapter 3, the application for licensure of a human monoclonal antibody against inhalational anthrax by Human Genome Sciences is on hold pending additional studies. One of the concluding remarks of the FDA-appointed committee of experts was that "there was no study with the antibiotic as the control arm" (FDA 2009b). While standard clinical care against inhalational anthrax is primarily the administration of antibiotics, the implication is that it should be a necessary component of the animal efficacy trial, both as a separate arm of the trial and as a combination treatment with the test product. The same trial design should apply when the standard of care in humans is supportive therapy alone.

expanded study design can be carried out in keeping with GLP principles. An example of this approach would be to combine body-temperature monitoring via a standard operating procedure in anticipation of fever as a clinical sign and initiation of antipyretic therapy in accordance with that standard operating procedure when the body temperature rises above a predetermined threshold as documented in the veterinary literature.

The Committee recognizes that the above is a different approach to using animals and generating data from animal trials. It is meant to be more comprehensive and apply considerations from human clinical trials (or treatment in the field) to animal studies in order to make more meaningful contributions to the interpretation of data as might be applied to humans. This strategy, i.e., provision of supportive care to animals subjected to severe disease, is not only more humane but may allow fewer animal numbers to be used in accordance with the principles of the Three Rs, as these are among the common causes of reduced validity of animal studies presented in Chapter 2, Table 2-9. Even though these actions can be performed in multiple grades of moderation without converting the laboratory into an intensive care unit, the practical difficulties of establishing this methodology in biocontainment facilities indicate the need for careful deliberation and study of the basic principles of such an approach and the creation of guidelines for the care and use of animals in research done under biocontainment conditions. The safety requirements of working in a biocontainment environment and the potential increased costs of implementing such types of animal trials are of considerable importance, especially for long-term studies.

IN VITRO TOOLS AND REPLACEMENT STRATEGIES

In the United States, a discussion of the future of the field of toxicology was prompted by the National Research Council report *Toxicity Testing in the 21st Century: A Vision and A Strategy* (NRC 2007). In 2008, several U.S. agencies, including the FDA, announced a coalition to facilitate this reports' implementation: "We propose a shift from primarily in vivo animal studies to in vitro assays, in vivo assays with lower organisms, and computational modeling for toxicity assessments" (Collins et al. 2008, p 906).

In Vitro and In Silico Methods

Biothreat agents are prime candidates for accelerated development and regulatory clearance of countermeasures by using animal models as alternatives to human clinical trials. This acceleration suggests the need to develop new and innovative strategies for collecting data and observations about how humans respond to these pathogens. Without this information the relevance of the animal models cannot be adequately ascertained. The same need exists for information obtained from the animal models to help develop and interpret new *in vitro* and computational *in silico* (IV/IS) methods. Advances in molecular characterization and in computational power have made it possible to consider approaches that do not even require the use of living systems, or at a minimum, accelerate the capacity of these artificial systems.

Many of the elements for a fully integrated IV/IS product development and approval strategy exist today, especially for anti-infectives against known agents that can be cultured in vitro. Standard techniques include high-throughput screening for drug discovery, in vitro testing of antimicrobial efficacy and drug resistance in many bacterial and viral pathogens, testing of some aspects of toxicity and pharmacokinetics and pharmacodynamics and computer modeling of structure-activity relationships. While in vitro assays for preclinical toxicity testing have been used extensively for several decades (reviewed in Judson et al. 2010), reliable assays for systemic toxicities, although improving,

remain a challenge (Adler et al. 2011; Hartung et al. 2011). Similarly, advances in computational capacity allow for increasingly complex modeling, but the animal models remain a critical bridge to test and confirm biosignatures (biomarkers) and other effects identified through studies of the natural history of the disease, relevant human clinical trials, or other animal model work. These biosignatures and pathways so identified and tested could then be a bridge to IV/IS models. However, despite technological advances the absence of suitable high-quality comparative data impedes the realization of this process (see previous discussion in Chapters 2 and 4 on the importance of human data).

The use of biomarkers in drug discovery and development is a related nascent area with great promise (pharmacogenomics; Hamburg and Collins 2010).[13] Suitable biomarkers, such as gene polymorphisms or gene expression profiles, can be determined in vivo (in animal models, natural infections, or clinical trials), and be used to predict whether a new candidate therapeutic is likely to be effective or, based on markers associated with adverse events, which individuals might be at increased risk for adverse reactions. This approach of moving from in vivo identification to in vitro testing of biomarkers has been used in the last few years for several biodefense-related agents, including monkeypox and anthrax. Time course studies of biomarker expression (i.e., gene expression arrays) following experimental infection of animals suggest that it may be possible (at least in some situations) to determine how long the patient has been infected, and whether optimal treatment varies depending on time after infection (Alkhalil et al. 2010; Das et al. 2008).

The immune system is a prime example of complex (and still incompletely understood) interactions of multiple cell types not yet amenable to IV/IS testing or modeling. An "artificial" functional in vitro immune system could facilitate the identification of candidate vaccines and therapeutics for immunosuppression or immune enhancement and could also help eliminate candidate therapeutics with undesirable immunologic properties. Although this work is still in the nascent stages, progress has been reported (Gaucher et al. 2008; Schanen and Drake 2008). In theory, this system, which depends on cell migration and maturation, will mimic what occurs in vivo and its output will more reliably reflect anticipated outcomes. Once created, this engineered tissue system can be manipulated and dynamic endpoints determined. For example, the Modular Immune In Vitro Constructs (MIMIC®) System, a simulated human immune system, enables testing of the adaptive immune response to vaccine antigens directly on microtiter plates and can provide multiple replicates of immune system activation and response from a single individual to different antigens ex vivo (Higbee et al. 2009). In recent years, a number of cell assemblages have been "engineered" in vitro to functionally mimic corresponding organs in the body (Fernandez and Khademhosseini 2010; Ingber et al. 2006; Mammoto and Ingber 2010). For instance, the development of micropatterned cocultures of human hepatocytes and supportive stromal cells permitted the growth of *Hepatitis C* virus (HCV) in vitro for the first time and, serving as a high-throughput platform, could allow rapid in vitro screening of candidate anti-HCV therapeutics for both efficacy and toxicity (Ploss et al. 2010). However, for this technology to assist in the development of countermeasures, the system must demonstrate that it accurately reflects human infections by using pathogens for which large amounts of (preferably) human in vivo data exist to test its reliability. Until such data are available, the system may be used to explore differences between multiple species (including humans) to further refine animal models or point to more accurate in silico representations of the human system.

[13] The term "pharmacoepigenetics" is sometimes used when gene expression, rather than DNA gene sequence variants, is the biomarker (Baer-Dubowska et al. 2011).

Computational Modeling

At present, computational approaches are most useful in the basic science phase of drug and vaccine development, specifically in identifying targets and biomarkers. These approaches are often helpful in the selection of the appropriate animal model because they allow the pathology and response to an agent to be defined in great detail. In this context, the computational approaches reduce animal testing by focusing future studies on the most promising leads and potentially by identifying biomarkers to develop humane endpoints for follow-up studies.

In their current form, IV/IS tools and strategies cannot serve as complete replacements for animal models. For complete replacements to be possible, it will be necessary to further define the functional and regulatory networks within the mammalian host and develop modeling approaches that allow prediction of how those networks (and ultimately the host's physiology) will behave when perturbed by infection or toxin. For infectious diseases, interactions of significance comprise pathogen and host responses, including the role of specific and nonspecific immune responses. The development of the first protease inhibitor against HIV-1 was a dramatic advance about two decades ago, and is probably the best known example of a new therapeutic developed by computational methods from structural information alone, but the hope is for many more examples in the future as both structural biology and computational expertise advance (Miller et al. 1989; for a general review on computer-aided drug design see Talele et al. 2010).

More recently, computational modeling has played an important role in the development of vaccines against influenza. For instance, the identification of highly conserved epitope sequences of the influenza virus elicited broadly reactive neutralizing antibodies that are currently pursued as potential "universal" influenza vaccines (Ekiert et al. 2010; Fleishman et al. 2011; Kang et al. 2011; Toussaint et al. 2011; Wang et al. 2010). Further, the identification of preexisting, cross-reacting epitopes against H1N1 viruses on human T-cells were used to test candidate vaccines against not only influenza viruses but other pathogens as well (Schanen et al. 2011).

In Vivo Tools to Improve Efficacy Testing

Surrogate animal models: smallpox

Orthopoxviruses are large DNA viruses that can infect a variety of vertebrate animals. Interestingly, a strong tropism effect is observed among members of the family *Orthopoxviridae*; thus, in most cases, a given orthopoxvirus infects only one host. Smallpox, caused by the variola virus, is an extremely virulent respiratory infection observed only in humans. Monkeypoxvirus infects a number of animal species, one of which is nonhuman primates. One of the primary virulence strategies observed during both of these infections is the generation of a large variety of viral immunomodulator proteins that prevent the host from mounting a protective immune response (Smith 1999).

Today smallpox is eradicated and no new infections of humans occur anywhere in the world (WHO 2011). Although a large amount of clinical information, including autopsy data, is available from past epidemics, the available scientific methods of the times did not allow for evaluation of the host response; thus comparison of mechanistic data with information obtained from current animal models is limited. Because of the stringent tropism effects, it is very difficult to infect animals with variola virus. One alternative method to overcome this hurdle is to create a surrogate animal model in which to establish, through the use of a different orthopox virus, similar pathophysiology and clinical disease to that observed in humans with smallpox. Jahrling and colleagues (2004) developed a non-human primate model of variola through the introduction of high doses (10^8 plaque-forming units; PFU) of virus intravenously and effectively bypassing the initial oropharyngeal site of virus replication. Due to the very limited and tightly controlled nature of variola virus research permitted by the World Health

Organization, further refinements of this model are difficult and expensive to achieve, although efforts are still ongoing to improve that model. To that effect, Hooper and colleagues (2004) proposed that a nonhuman primate model of monkeypox given infective doses that closely represent the typical exposure to this virus, would be more relevant to and accurately present smallpox than the use of nonphysiological doses and routes of infection developed with variola virus. One reason for the potential effectiveness of this strategy is the ability of the orthopoxviruses to produce strong, cross-reactive immune responses in animals of different species. An additional benefit of this strategy may be the relevance to "newer" types of human orthopoxvirus diseases, such as human monkeypox, which have emerged as serious public health burdens in places where endemic smallpox was observed in the past (Rimoin et al. 2010). Animals of several species can serve as natural reservoirs for the monkeypox virus (Khodakevich et al. 1986, 1987a, 1987b); in this case, using surrogate animal models susceptible to monkeypox virus would be a more natural approach than the persistent use of variola virus on organisms with no natural affinity for this agent. Moreover, because the tropism effect is likely to occur with other pathogens, the monkeypox strategy may become a paradigm with future use as well (McFadden 2005).

Systems approaches to infectious diseases

Virtually all human diseases are a manifestation of interactions among many inherited polymorphic genes and environmental factors (Churchill et al. 2004; Cookson et al. 2009; Kotb 2010; Kotb et al. 2008; Thompson 1995; Villar et al. 2004; Voit et al. 2008; Williams 2006). Traditional reductionist approaches to develop disease models based on gene-by-gene comparisons or extrapolations have been universally applicable. Broader systems approaches may be useful in this regard because they can reveal how disease variables influence one another within a whole organism; provide a roadmap to expedite the discovery of networks of pathways that modulate disease susceptibility and outcomes; and reveal those networks likely to be good candidates for the development of more targeted rapid diagnostics and effective therapeutics.

Although animals with limited genetic diversity have several advantages (see above), translating findings from these animals to humans is not always useful. Whereas nonhuman primates offer sufficient genetic variation for the implementation of a systems perspective (Sasaki et al. 2009; Wolfe et al. 1998), the number of replicate studies needed to generate these data is limited by ethical considerations, inadequate stocks, and prohibitive cost. Inbred rodents, although useful for generating the quantity of data needed for systems evaluation, are characterized by little genetic heterogeneity. To address these challenges, novel animal models have been developed from which discoveries, made with a systems genetics or biological approach, are likely to translate to humans more readily. For example, recombinant inbred mice (Advanced, or the next generation Collaborative Cross strains) are generated and bred to maximize the number of recombinations in each of their chromosomes thereby diversifying their genetic context and exposing a wider spectrum of disease phenotypes (Durrant et al. 2011; Kotb 2010; Williams et al. 2001).

When infected, these strains exhibit a wide spectrum of disease phenotypes because, as is the case in humans, random assortment of many polymorphic loci can accentuate resistance or susceptibility to a particular disease. Accordingly, findings in these genetically diverse populations can significantly enhance the translation of experimental research findings to the clinical setting to prevent or improve the management of complex infectious diseases. Network-based systems approaches and pathway-to-pathway comparisons between species are now more likely to expedite the discovery of targets and networks and the translation of research across species than gene-by-gene comparisons (for other comparative biological approaches see discussion on compartmentalization, Chapter 4, p 56).

PROMISES AND CHALLENGES FOR THE FUTURE

FDA Commissioner Margaret Hamburg recently wrote "We must bring 21st century approaches to 21st century products and problems" (Hamburg 2011). This is a time of rapid and unprecedented development of enabling biotechnologies that hold great promise for the future, but it also presents several serious challenges. Despite the diversity of currently available approaches and promising technologies, no approaches can at this time fully address the shortfalls of using animal models as complete surrogates for humans. The Committee notes the following concerns:

- As stated in Chapters 2 and 4, there is a need to develop new and innovative strategies for collecting data about how humans will respond to pathogens of concern. Without this information, there can be little useful comparison to animal models (or qualification thereof, see Chapter 4), the effectiveness and predictability of biomarkers is curtailed, and the animal data to be used for the development and interpretation of meaningful IV/IS methods will not be accurate. Further, original data (positive or negative; human and animal) may not be systematically shared with the wider research community (as also discussed in Chapter 3, p 44). The lack of sharing causes the fragmentation of knowledge and prevents the comparison of inputs and outcomes,[14] which may be particularly important in the event of an "unknown-unknown" emergency. Therefore, this information should be collected systematically, consistently, and accurately and be made available to the research community to enable progress toward standardization of methods and qualification of models, and to address ethical concerns regarding the potential nonproductive or duplicative use of animals or the unnecessary duplication of studies and waste of resources.
- The provision of supportive veterinary care during animal efficacy trials for countermeasures is a means to improve data gathering from animal models to enhance the efficiency and productivity of this research field. In the *Draft Guidance for Industry* the FDA states that "studies should be designed to mimic the clinical scenario and achieve meaningful outcomes comparable to the endpoints desired in humans. In some instances, supportive care should be administered to the animals as part of the study design" (FDA 2009). The Animal Rule does not require that a test product exhibit added benefit over conventional therapy ("…the drug product is reasonably likely to produce clinical benefit in humans."; FDA 2002, p 37995), but if conventional therapy is beneficial for human patients then it is a reasonable measure to include in the study design. Furthermore, studies that include provision of the standard of care as one arm were suggested at the public meeting to evaluate the licensure application of raxibacumab under the Animal Rule (FDA 2009b). Since for most countermeasures in development there is no other standard of care than supportive therapy, it is appropriate to include it when evaluating the test products. Experience with such study designs and experimental protocols may be helpful in the event of an efficacy trial for a countermeasure against an "unknown-unknown". **Due to the nature of biocontainment, defining the basic principles of such an approach —including guidelines for the care and use of animals in research done in biocontainment facilities— is recommended**.

[14] In addition to data sharing being one of the principle tenets of responsible conduct of research (see the Office of Research Integrity's Introduction to the Responsible Conduct of Research, http://ori.hhs.gov/education/products/RCRintro/), it also is a fundamental tool of "the economy of knowledge production" (Nat Genet 2011).

- The Public Health Emergency Medical Countermeasures Enterprise (PHEMCE) Review emphasized the primary role for regulatory science[15] in biodefense research (DHHS 2010). As shown in Figure 5-1, a window of opportunity may exist in which regulatory science can help to overcome the limited use of advanced in vitro and in vivo technologies in the development of medical countermeasures. It is desirable to develop criteria for choosing the most suitable methods, and essential to do this in a way that will allow effective utilization of IV/IS technologies while not inhibiting advances. Steps in the product development process have a clear potential use for IV/IS as an adjunct method but the use of the whole animal will not be replaced in the process. A research strategy to address these gaps would be useful as well as improve areas in which in vitro assays are already showing promise. **A place to begin would be an analysis of the discovery, development, and approval process for medical countermeasures to identify (1) where the most important scientific gaps exist in terms of utilizing alternative methods to animal models and how to address them; (2) the specific areas where the use of in vitro and in silico methods could be sufficient or as an adjunct to the use of animals; and (3) the criteria for choosing and utilizing the most suitable technologies to replace animal use in biodefense research.**
- Regulations that require humane treatment of animals in research (such as the AWA as discussed above) do not impose principled limits on the use of animals, i.e., pain and distress caused by the research protocols are to be minimized only when and to the extent consistent with the needs of science (Walker and King 2011). However, **the needs of science in this research field should be weighed against the potential advances in knowledge and benefits to the warfighters as well as against the duration and severity of animal pain and distress**. In previous sections, the report outlines the need for the development of humane endpoints and biomarkers, for the administration of supportive clinical care, and for the alleviation of pain and distress. Medical countermeasures research and development for biodefense currently depends on the continued use of nonhuman primates, as discussed in chapter 2, and will probably remain so until such time that robust alternatives (either absolute or relative) to their use are available.[16] However, the report's conclusions and recommendations could help reduce a key tension in animal research, namely that the animals that most resemble humans are simultaneously viewed as most necessary for research that is impermissible in humans and as having greater moral value because they resemble humans. The recommended comprehensive strategy of implementing the Three Rs, utilizing compartmentalization and systems biology, and enhancing collection and analysis of human data reduces dependency on nonhuman primates by maximizing the value of data derived from all research.[17] **The Committee recommends that, where possible, the TMT should encourage efforts to replace nonhuman primates as the animal of choice in biodefense research.** In addition, unhindered access to data (as discussed above) and publishing of all results —including negative ones— are critical steps to ensure that this data is indeed useful, animals are used judiciously, and unnecessary duplication of work is avoided (Bateson 2011).

[15] "The development and use of new tools, standards, and approaches to more efficiently develop products and to more effectively evaluate product safety, efficacy, and quality" (FDA 2010).

[16] The authors of the recent *Review of Research Using Non-Human Primates* "agreed that in many cases the use of NHPs was justifiable even in the context of current understanding of animal welfare and advances in knowledge that might now render some work on living animals unnecessary" (Bateson 2011, p 1).

[17] To cite from the *Review of Research Using Non-Human Primates*, "it is an ethical imperative that maximum benefit be derived from studies employing NHPs" (Bateson 2011, p 3).

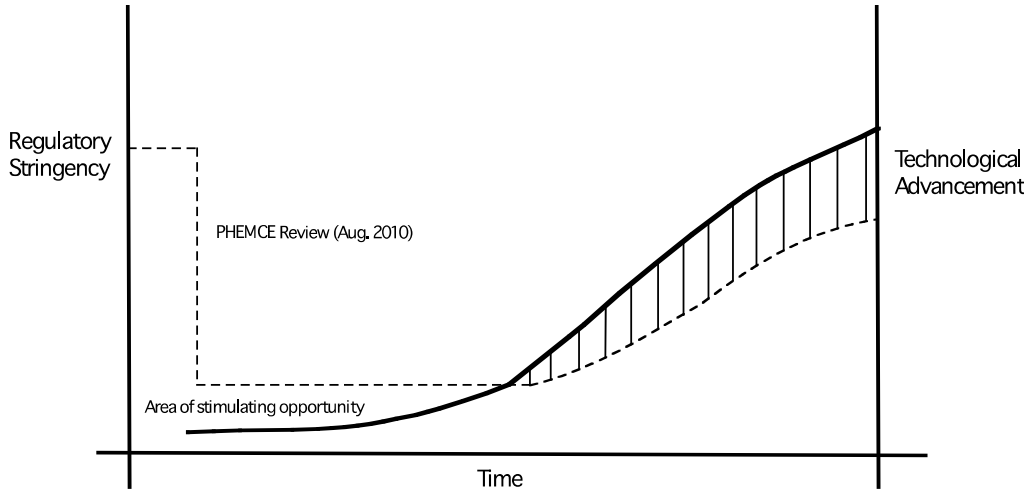

FIGURE 5-1 Regulatory science proceeds as a function of regulatory stringency and technological advancement. Whereas stringency is necessary to safeguard the safety and efficacy of products, it can be better achieved as newer technologies and reliable models provide a better approximation of the human system (or a relevant component of the human system). Greater innovation or investment in many of the suggested approaches above may be achieved by adjusting the real or perceived stringency of the current regulatory framework. As technologies, models or approaches are discovered that provide better fidelity with a human system (or the relevant component of the human system), then standardization may be achieved and stringency increased based on demonstration of the model's reliability.

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Appendix A

The Animal Rule
21 CFR Parts 314 and 601

d.6. Silicon carbide;
d.7. Tantalum or tantalum alloys;
d.8. Titanium or titanium alloys;
d.9. Titanium carbide; *or*
d.10. Zirconium or zirconium alloys.
e. Distillation or absorption columns of internal diameter greater than 0.1 m, and liquid distributors, vapor distributors or liquid collectors designed for such distillation or absorption columns, where all surfaces that come in direct contact with the chemical(s) being processed are made from any of the following materials:
e.1. Alloys with more than 25% nickel and 20% chromium by weight;
e.2. Fluoropolymers;
e.3. Glass (including vitrified or enamelled coatings or glass lining);
e.4. Graphite or carbon-graphite;
e.5. Nickel or alloys with more than 40% nickel by weight;
e.6. Tantalum or tantalum alloys;
e.7. Titanium or titanium alloys; *or*
e.8. Zirconium or zirconium alloys.
f. Remotely operated filling equipment in which all surfaces that come in direct contact with the chemical(s) being processed are made from any of the following materials:
f.1. Alloys with more than 25% nickel and 20% chromium by weight; *or*
f.2. Nickel or alloys with more than 40% nickel by weight.
g. Valves with nominal sizes greater than 1.0 cm (3/8 in.), in which all surfaces that come in direct contact with the chemical(s) being processed or contained are made from any of the following materials:
g.1. Nickel or alloys with more than 40% nickel by weight;
g.2. Alloys with more than 25% nickel and 20% chromium by weight;
g.3. Fluoropolymers;
g.4. Glass or glass lined (including vitrified or enameled coatings);
g.5. Tantalum or tantalum alloys;
g.6. Titanium or titanium alloys; *or*
g.7. Zirconium or zirconium alloys.
h. Multi-walled piping incorporating a leak detection port, in which all surfaces that come in direct contact with the chemical(s) being processed or contained are made from any of the following materials:
h.1. Alloys with more than 25% nickel and 20% chromium by weight;
h.2. Fluoropolymers;
h.3. Glass (including vitrified or enamelled coatings or glass lining);
h.4. Graphite or carbon-graphite;
h.5. Nickel or alloys with more than 40% nickel by weight;
h.6. Tantalum or tantalum alloys;
h.7. Titanium or titanium alloys; *or*
h.8. Zirconium or zirconium alloys.
i. Multiple-seal, canned drive, magnetic drive, bellows or diaphragm pumps, with manufacturer's specified maximum flow-rate greater than 0.6 m^3/hour, or vacuum pumps with manufacturer's specified maximum flow-rate greater than 5 m^3/hour (under standard temperature (273 K (0° C)) and pressure (101.3 kPa) conditions), and casing (pump bodies), preformed casing liners, impellers, rotors or jet pump nozzles designed for such pumps, in which all surfaces that come into direct contact with the chemical(s) being processed are made from any of the of the following materials:
i.1. Alloys with more than 25% nickel and 20% chromium by weight;
i.2. Ceramics;
i.3. Ferrosilicon;
i.4. Fluoropolymers;
i.5. Glass (including vitrified or enamelled coatings or glass lining);
i.6. Graphite or carbon-graphite;
i.7. Nickel or alloys with more than 40% nickel by weight;
i.8. Tantalum or tantalum alloys;
i.9. Titanium or titanium alloys, *or*
i.10. Zirconium or zirconium alloys.
j. Incinerators designed to destroy chemical warfare agents, chemical weapons precursors controlled by 1C350, or chemical munitions having specially designed waste supply systems, special handling facilities and an average combustion chamber temperature greater than 1000°C in which all surfaces in the waste supply system that come into direct contact with the waste products are made from or lined with any of the following materials:
j.1. Alloys with more than 25% nickel and 20% chromium by weight;
j.2. Ceramics; *or*
j.3. Nickel or alloys with more than 40% nickel by weight.

Technical Note: Carbon-graphite is a composition consisting primarily of graphite and amorphous carbon, in which the graphite is 8 percent or more by weight of the composition.

19. In Supplement No. 1 to Part 774 (the Commerce Control List), Category 2—Materials Processing, is amended by revising the List of Items Controlled section in ECCN 2B352 to read as follows:

2B352 Equipment capable of use in handling biological materials, as follows (see List of Items Controlled).

* * * * *

List of Items Controlled

Unit: Equipment in number.
Related Controls: N/A.
Related Definitions: For purposes of this entry, isolators include flexible isolators, dry boxes, anaerobic chambers and glove boxes.
Items:
a. Complete containment facilities at P3 or P4 containment level.

Technical Note: P3 or P4 (BL3, BL4, L3, L4) containment levels are as specified in the WHO Laboratory Biosafety Manual (Geneva, 1983).

b. Fermenters capable of cultivation of pathogenic microorganisms, viruses, or for toxin production, without the propagation of aerosols, having a capacity equal to or greater than 100 liters.

Technical Note: Fermenters include bioreactors, chemostats, and continuous-flow systems.

c. Centrifugal separators capable of the continuous separation of pathogenic microorganisms, without the propagation of aerosols, and having all of the following characteristics:
c.1. One or more sealing joints within the steam containment area;
c.2. A flow rate greater than 100 liters per hour;
c.3. Components of polished stainless steel or titanium; *and*
c.4. Capable of *in situ* steam sterilization in a closed state.

Technical Note: Centrifugal separators include decanters.

d. Cross (tangential) flow filtration equipment capable of continuous separation of pathogenic microorganisms, viruses, toxins, and cell cultures without the propagation of aerosols, having all of the following characteristics:
d.1. Equal to or greater than 5 square meters;
d.2. Capable of *in situ* sterilization.
e. Steam sterilizable freeze-drying equipment with a condenser capacity of 10 kgs of ice or greater in 24 hours, but less than 1,000 kgs of ice in 24 hours.
f. Protective and containment equipment, as follows:
f.1. Protective full or half suits, or hoods dependant upon a tethered external air supply and operating under positive pressure;

Technical Note: This entry does not control suits designed to be worn with self-contained breathing apparatus.

f.2. Class III biological safety cabinets or isolators with similar performance standards, *e.g.,* flexible isolators, dry boxes, anaerobic chambers, glove boxes or laminar flow hoods (closed with vertical flow).
g. Chambers designed for aerosol challenge testing with microorganisms, viruses, or toxins and having a capacity of 1 m^3 or greater.

Dated: May 23, 2002.

James J. Jochum,

Assistant Secretary for Export Administration.

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BILLING CODE 3510–33–P

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

21 CFR Parts 314 and 601

[Docket No. 98N–0237]

RIN 0910–AC05

New Drug and Biological Drug Products; Evidence Needed to Demonstrate Effectiveness of New Drugs When Human Efficacy Studies Are Not Ethical or Feasible

AGENCY: Food and Drug Administration, HHS.

ACTION: Final rule.

SUMMARY: The Food and Drug Administration (FDA) is amending its new drug and biological product regulations to allow appropriate studies in animals in certain cases to provide

substantial evidence of the effectiveness of new drug and biological products used to reduce or prevent the toxicity of chemical, biological, radiological, or nuclear substances. This rule will apply when adequate and well-controlled clinical studies in humans cannot be ethically conducted and field efficacy studies are not feasible. In these situations, certain new drug and biological products that are intended to reduce or prevent serious or life-threatening conditions may be approved for marketing based on evidence of effectiveness derived from appropriate studies in animals and any additional supporting data.

DATES: This rule is effective July 1, 2002.

FOR FURTHER INFORMATION CONTACT:
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SUPPLEMENTARY INFORMATION:

I. Introduction

In the **Federal Register** of October 5, 1999 (64 FR 53960), we (FDA) proposed to amend our new drug and biological product regulations to identify the information needed to provide substantial evidence of the effectiveness of certain new drug and biological products used to reduce or prevent the toxicity of chemical, biological, radiological, or nuclear substances. We are finalizing that proposed rule by adding subpart I to part 314 (21 CFR part 314) and subpart H to part 601 (21 CFR part 601).

This final rule provides for approval of certain new drug and biological products based on animal data when adequate and well-controlled efficacy studies in humans cannot be ethically conducted because the studies would involve administering a potentially lethal or permanently disabling toxic substance or organism to healthy human volunteers and field trials are not feasible prior to approval. Under this rule, in these situations, certain new drug and biological products that are intended to reduce or prevent serious or life-threatening conditions can be approved for marketing based on evidence of effectiveness derived from appropriate studies in animals, without adequate and well-controlled efficacy studies in humans (§ 314.126). In assessing the sufficiency of animal data, the agency may take into account other data, including human data, available to the agency. Under this rule, FDA can rely on the evidence from animal studies to provide substantial evidence of the effectiveness of these products when:

1. There is a reasonably well-understood pathophysiological mechanism for the toxicity of the chemical, biological, radiological, or nuclear substance and its amelioration or prevention by the product;

2. The effect is demonstrated in more than one animal species expected to react with a response predictive for humans, unless the effect is demonstrated in a single animal species that represents a sufficiently well-characterized animal model (meaning the model has been adequately evaluated for its responsiveness) for predicting the response in humans;

3. The animal study endpoint is clearly related to the desired benefit in humans, which is generally the enhancement of survival or prevention of major morbidity; and

4. The data or information on the pharmacokinetics and pharmacodynamics of the product or other relevant data or information in animals and humans is sufficiently well understood to allow selection of an effective dose in humans, and it is therefore reasonable to expect the effectiveness of the product in animals to be a reliable indicator of its effectiveness in humans.

All studies subject to this rule must be conducted in accordance with preexisting requirements under the good laboratory practices (21 CFR part 58) regulations and the Animal Welfare Act (7 U.S.C. 2131 *et. seq.*).

Safety evaluation of products is not addressed in this rule. Products evaluated for effectiveness under subpart I of part 314 and subpart H of part 601 will be evaluated for safety under preexisting requirements for establishing the safety of new drug and biological products. The agency believes that the safety of most of these products can be studied in human volunteers similar to the people who would be exposed to the product. FDA recognizes that some safety data, such as data on possible adverse interactions between the toxic substance itself and the new product, may not be available. This is not expected to keep the agency from making an adequate safety evaluation. FDA's procedures and standards for evaluating the safety of new drug and biological products are sufficiently flexible to provide for the safety evaluation of products evaluated for efficacy under subpart I of part 314 and subpart H of part 601.

This rule will not apply if product approval can be based on standards described elsewhere in our regulations (for example, accelerated approval based on human surrogate markers or clinical endpoints other than survival or irreversible morbidity).[1]

II. Comments on the Proposed Rule and Our Response

We received comments on the proposed rule from two pharmaceutical companies and one physician affiliated with a university. We also received comments from the National Institutes of Health (NIH). The NIH comments were based on a prepublication draft of the proposed rule, but the comments were received too late to be addressed in the proposed rule. The NIH comments have been placed in the docket for this rule and are addressed in this document.

In addition to the changes we have made in response to comments, we have changed the titles of subpart I of part 314 and subpart H (formerly subpart G) of part 601 to better describe the scope of the subparts. Subpart I of part 314 is now entitled "Approval of New Drugs When Human Efficacy Studies Are Not Ethical or Feasible" and subpart H of part 601 is now entitled "Approval of Biological Products When Human Efficacy Studies Are Not Ethical or Feasible." Proposed subpart G has been redesignated as subpart H in the final rule because subpart G has since been designated for regulations on postmarketing studies. Proposed §§ 601.60 through 601.65 have been renumbered §§ 601.90 through 601.95 in subpart H.

We have also changed, on our own initiative, the requirements proposed in §§ 314.610(c) and 601.61(c) (§§ 314.610(b)(3) and 601.91(b)(3) in this final rule). We have deleted the requirement that self-administered drug products approved under this rule be in unit-of-use packaging with attached patient labeling. In addition, we have eliminated the distinction between self-

[1] An example of a drug approval based on human surrogate markers is our August 30, 2000, approval of an efficacy supplement for ciprofloxacin. Ciprofloxacin HCl was approved for postexposure management of inhalational anthrax. The approval was based, in part, on human studies demonstrating that ciprofloxacin achieved serum concentrations reaching or exceeding levels associated with improved survival of animals exposed to aerosolized *Bacillus anthracis* spores. The results from these studies were combined with the knowledge of effectiveness in humans of ciprofloxacin for other bacterial infections, including pneumonia. The validity of the human surrogate marker was supported by animal studies.

administered products and products administered by health professionals.

Whether a product is self-administered or administered by a health professional, it is important to inform patient recipients that a product approved under this rule has not been studied for efficacy in humans because of ethical or feasibility reasons.[2] It is also important that patient recipients receive information about indications, dosage and administration, contraindications, reasonably foreseeable risks, adverse reactions, anticipated benefits, and drug interactions. This rule requires that all of this information be provided to patient recipients of products approved under subpart I of part 314 and subpart H of part 601.

We believe, however, that the proposed unit-of-use packaging and attached patient-labeling requirement could have had the unintended effect of hampering the distribution and dispensing of these products in the event of an emergency. The added bulk of unit-of-use packaging could have made stockpiling and transporting more difficult in many cases. The proposed requirement might also have hampered the speedy distribution of products for additional indications previously approved outside of this rule.

Applicants may meet the requirements of new §§ 314.610(b)(3) and 601.91(b)(3) in a variety of ways, as long as sponsors make provisions to get the information to patients. For example, the sponsor could provide reproducible master copies of labeling information or presentations for patient recipients that would be appropriate in the event of an emergency.

We have also changed proposed §§ 314.610(c) and 601.61(c) (§§ 314.610(b) and 601.91(b) in this final rule) to require that the patient labeling explain that, for ethical or feasibility reasons, the product's approval was based on efficacy studies conducted only in animals. This explanation will better inform patient recipients about the nature and ethical basis of the product approval under this rule and how that approval differs from approval of products based on standard human efficacy studies.

Finally, we have added to §§ 314.610(b)(1) and 601.91(b)(1) (proposed §§ 314.610(a) and 601.61(a)) a requirement that applicants include a plan or approach to fulfilling postmarketing study commitments as part of their application. We recognize that such studies normally will not be conducted unless an emergency arises that requires the product's use. Furthermore, when the product is used in an emergency, it may not be feasible for sponsors to conduct postmarketing studies in a timely manner, nor is it our intention to require sponsors to send investigators into areas of exposure. We do, however, believe that applicants can plan a postmarketing study approach, in consultation with the agency, as part of an overall response to an event.

The requirement to submit a plan for postmarketing studies is consistent with the requirements for sponsors under the accelerated approval process provided for in subpart H of part 314.

The procedures in subpart H and in this rule are similar because, to assess efficacy, both allow use of an endpoint that is not a clinical endpoint showing a benefit. Instead the rules under subpart H allow for reliance on a clinical surrogate endpoint and this rule allows for the use of animal data as an endpoint.

Postmarketing studies are critical in both of these situations to verify and describe the clinical benefit of the drug or biological product. The postmarketing studies may provide us with data that directly verify that the product provides the desired benefit in humans, such as increased survival or prevention of major morbidity.

(Comment 1) One comment suggested that we define ''lethal'' and ''permanently disabling.'' The comment expressed concern that without such definitions, subpart I of part 314 and subpart H of part 601 will be misapplied in situations where clinical testing can and should be carried out.

The definitions of ''lethal'' and ''permanently disabling'' would seem to be well understood. Although we share the concern that too expansive an interpretation of ''lethal'' or ''permanently disabling'' could lead to attempts to apply this rule when human studies are, in fact, feasible, we are also concerned that too restrictive a definition of ''lethal'' or ''permanently disabling'' could lead to failure to apply subpart I of part 314 and subpart H of part 601 in situations where they should be applied to protect the public health. We believe that, as a general matter, we must rely on the good sense and responsibility of those health professionals who will be seeking to apply subpart I of part 314 and subpart H of part 601 in the future, and on responsible review of specific cases by FDA. Nevertheless, we can provide guidance for applying subpart I of part 314 and subpart H of part 601 by clarifying that a ''lethal substance'' is one that is likely to kill at least some of the humans who have been exposed to the substance and a ''permanently disabling substance'' is one that is likely to cause a permanent physical or mental impairment that substantially limits one or more of the major life activities in at least some of the humans who have been exposed to the substance.

(Comment 2) One comment stated that the rule does not explicitly cover infectious substances and pointed out that not all infectious substances produce toxins. The comment suggested replacing ''toxic'' with ''toxic and/or infectious'' in proposed §§ 314.600 and 601.60 (§ 601.90 in this final rule).

The rule is certainly intended to cover products for treatment of infections. At some level, an infectious agent that is lethal or permanently disabling is toxic to its host, even if that agent is not itself a ''toxin'' or a producer of ''toxins'' within a strict definition of the word. Because we do not use ''toxin'' in the rule, and ''toxic'' is accurate, we do not believe we need to replace ''toxic'' with ''toxic and/or infectious'' to indicate that products for the treatment of infections may be approved under this rule.

(Comment 3) One comment noted that the proposed rule did not discuss criteria that should be applied in determining if ''an important medical need is not adequately met by currently available therapies.'' The comment suggested that we state that we will use the criteria given in our guidance for industry entitled ''Fast Track Drug Development Programs—Designation, Development, and Application Review'' (September 1998).

We have decided to eliminate the requirement that ''products would be expected to provide meaningful therapeutic benefits to patients over existing treatments,'' as well as the limitation that the toxic agent be ''without a proven treatment'' (proposed §§ 314.600 and 601.60). Recent events involving the multiple exposures to anthrax in our population, and deaths resulting from those infections, have indicated a need for a wide range of therapeutic options that, in some instances, might be inappropriately limited by requiring new products to have a therapeutic benefit over existing treatments, or to be used only in the absence of a proven treatment. Availability of a variety of drug and biological products is important because, for example, patient recipients may be allergic to one product and require another, may be intolerant of a product because of side effects, or may respond more favorably to one product

[2] In some cases, however, such as with anti-infective drug products, it would usually be expected that human data on safety and effectiveness for other indications may be available.

than another. We also believe that a wider variety of therapeutic choices will limit potential problems with availability, accessibility, and distribution of products. We have modified the final rule to address these concerns and help ensure the availability of more than one therapeutic option.

(Comment 4) One comment requested that antivenin and antitoxin products of animal origin be considered for inclusion specifically on the list of new drugs and biological products to which the rule applies.

There is no list of products that may be approved based on evidence of effectiveness from efficacy studies in animals. The rule provides criteria to determine if evidence of effectiveness from efficacy studies in animals may support approval of a product. If an antivenin or antitoxin product of animal origin meets the criteria specified in the rule, it may be approved on the basis of evidence of effectiveness from efficacy studies in animals.

(Comment 5) One comment requested that we revise proposed §§ 314.610 and 601.61 (§ 601.91 in this final rule) to state that substantiation in multiple animal species is required only where appropriate. The comment stated we should not limit ourselves to approvals only when there is substantiation in ''multiple'' animal species. The comment contended that where independent studies in a single species meet the general principles of independent substantiation as described in the guidance for industry entitled ''Providing Clinical Evidence of Effectiveness for Human Drugs and Biological Products'' (May 1998), those studies are sufficient to substantiate effectiveness as a matter of science and a requirement of substantiation in multiple species would result in an unnecessary delay of agency approval. According to the comment, these concerns are particularly important where viruses have a narrow host range and conducting efficacy trials in more than one animal species in such cases either is not feasible or provides only limited additional information that is relevant to the full-blown disease in humans. The comment suggested that the requirement of substantiation in multiple species in a given case should depend on the known host range and the availability of animal model systems.

We share some of the concerns expressed in the comment, but we believe the proposed remedy goes too far. Approval of the use of a drug lacking human evidence of effectiveness represents a significant departure from ordinary practice. There are countless examples of treatments with favorable effects in animals that did not prove effective in humans. Although this rule does, for good reason, allow reliance on animal studies when human studies cannot be conducted, in general we expect that the evidence, to be persuasive, should be developed in more than one animal species unless the effect is demonstrated in a single animal species that represents a sufficiently well-characterized animal model for predicting the response in humans. We recognize that conducting studies in more than one species can result in added expense, but we believe this is warranted because of the additional assurance they would provide.

Furthermore, reliance on our guidance entitled ''Providing Clinical Evidence of Effectiveness for Human Drugs and Biological Products'' is misplaced. That guidance was drafted to provide advice on the quantity of data from clinical studies needed to support a finding of effectiveness and, specifically, on when the agency ought to rely on a single human study. The guidance addressed cases in which the issue is the credibility of the data itself, not the relevance of the data to humans. In this rule, the issue is the ability of results from animal studies to predict the human response, and not the credibility of the animal finding itself (although, of course, the animal studies should be replicated or substantiated in each species as needed to ensure credible results). The need for multiple species in certain cases is to enhance the likelihood that the data are pertinent to humans.

We do recognize, however, that the multiple species requirement could be inappropriate or unnecessary in certain situations. For example, there may be only one species capable of reacting with a response predictive for humans. This would occur where there is only one nonhuman host for the targeted microorganism. There may also be other situations in which studies in a particular species are specifically well recognized as predictors of effectiveness in humans. Thus, circumstances in which the agency will rely on evidence from studies in one animal species to provide substantial evidence of the effectiveness of these products in humans would generally be limited to situations where the study model is sufficiently well-recognized so as to render studies in multiple species unnecessary. In addition, other human data for the product could provide support for such approvals.

Accordingly, we have changed proposed §§ 314.610 and 601.61 (§ 601.91(c) in this final rule) to require that approval be based on studies in more than one animal species unless the effect is demonstrated in a single animal species that represents a sufficiently well-characterized animal model for predicting the response in humans. The agency believes that demonstrating effectiveness in studies conducted in a single animal species using a well-characterized animal model will most often be done for anti-infective drug products. The pathophysiological mechanisms of infectious diseases are usually very well understood, and animal models for many infectious diseases have been studied for years and are very well characterized.

(Comment 6) One comment suggested we remove the requirement that there be a reasonably well-understood pathophysiological mechanism of the toxicity of the substance and its prevention or substantial reduction by the product. The comment stated it is hard to say when we understand something reasonably well and that, if we decide to retain the requirement, we should state at what level (e.g., cellular, molecular) the mechanism must be understood.

A disease's or toxin's mechanism of action does not need to be understood before a safe and effective treatment or preventative can be devised. Quinine and Jenner's smallpox vaccine were both developed before the acceptance of the germ theory of disease. Neither is there a general requirement that an applicant who is relying on human testing to establish effectiveness demonstrate the mechanism of action of the drug or biological product that is the subject of the marketing application. It is generally sufficient to demonstrate that a product is safe and effective. It is generally not required that an applicant demonstrate how or why the product is safe and effective.

It is true that a pathophysiologic understanding of a disease and treatment is not required when human studies are used to support approval. In the case of human drug or biological products approved on the basis of evidence of effectiveness from studies in animals, however, we are requiring an understanding of the mechanism of the toxic substance or infectious organism and its prevention or reduction by the product. This understanding helps provide assurance that the efficacy data from studies in animals can be applied to humans. We have not specified exactly what degree of pathophysiologic understanding is needed, and that will be a matter of judgment. The level of understanding could range from a complete understanding of how a toxic

substance works at the cellular level in both human and animal cells together with a clear understanding of what the antidote does at the molecular level to a less complete understanding. The level of required understanding of the mechanism of action of the toxic substance or infectious organism and the product may vary from toxic substance to toxic substance or infectious organism to infectious organism and could even vary from one product to another intended to treat the same condition.

(Comment 7) One comment suggested that an institutional review board (IRB) or other ethical scientific review body determine if it would be unethical to conduct studies in humans. The comment also said we do not mention who would make the determination that it would be unethical to conduct studies in humans.

The final determination that it is unethical to conduct studies in humans will be made by the reviewing officials in FDA. We anticipate that in most cases the determination as to whether it would be unethical to conduct studies in humans will not be difficult. In those cases that are difficult, the views of one or more IRBs, individual ethicists and clinicians, and FDA advisory committees could be sought by a sponsor or FDA. A case where such a consultation could be useful is one in which a putatively subtoxic dose would be used in humans to establish at least a mechanism for protection, if not actual protection.

(Comment 8) One comment noted that we said in the proposed rule:

> The agency also intends in most cases to consult on applications to market such products with an advisory committee, supplemented with appropriate expert consultants, in meetings open to the public in order to receive expert advice on whether a particular set of animal data support efficacy of a product under this rule (64 FR 53960 at 53964 and 53965).

The comment asked us to consider requiring consultation with an advisory committee either before conducting the animal studies or before approval of the product, or both.

We want to reiterate our statement in the proposed rule that we intend usually to consult with an advisory committee during the approval process. Indeed, we may consult with an advisory committee more than once on a single product if circumstances warrant it. Consultation with an advisory committee could occur early in the development process, to discuss whether the concept of using certain animal data to support efficacy is reasonable.

Even though consultation with an advisory committee is generally desirable, it is not always practical. For example, products reviewed under this rule may be part of the response to a public health emergency; therefore, there may not be time to convene an advisory committee. Accordingly, we believe that it would be inappropriate to absolutely require consultation with an advisory committee.

(Comment 9) One comment questioned whether patient labeling is adequate to inform patients that a product has been approved on the basis of animal efficacy data, particularly in situations where military personnel are ordered to take a product approved under this rule. The comment did not suggest an alternative to the provisions of the rule.

Sections 314.610(b)(3) and 609.91(b)(3) provide that for products or specific indications approved under this rule, applicants must prepare, as part of their proposed labeling, labeling to be provided to patients or potential patients. The patient labeling, written in language that can be easily understood by the general public, must explain that, for ethical or feasibility reasons, the product's approval was based on efficacy studies conducted in animals alone. The labeling must give the product's indication(s), directions for use (dosage and administration), contraindications, a description of any reasonably foreseeable risks, adverse reactions, anticipated benefits, drug interactions, and any other relevant information required by FDA at the time of approval. If possible, the patient labeling must be available with the product to be provided to patients or potential patients prior to administration or dispensing of the product for the use approved under this rule. We intend that in interpreting §§ 314.610(b)(3) and 601.91(b)(3), the word "possible" be given its ordinary and literal meaning. Situations in which it would be inconvenient or require some effort to make the labeling available for patients should not be equated with situations in which it would be impossible to do so.

These provisions, coupled with communications within a health care provider-patient relationship should, as a general matter in both civilian and military contexts, adequately ensure that patients are informed that the product they are taking has been approved based on animal efficacy data.

(Comment 10) One comment suggested that labeling a drug or biological product approved on the basis of evidence of effectiveness from studies in animals as "FDA approved" is misleading, because patients would assume that the product had been approved based on human studies. The comment suggested that we treat the product as an investigational new drug, but waive certain requirements generally applied to investigational new drugs, if those requirements would provide obstacles to the product's use in an emergency.

We agree that the labeling would be misleading if information were not included to explain to patients or potential patients that the effectiveness of the product was demonstrated in animals not humans, and that this reliance on animal efficacy data was based on ethical and feasibility concerns. Therefore, under sections 502(a) and 701(a) of the Federal Food, Drug, and Cosmetic Act (the act) (21 U.S.C. 352(a) and 372(a)) (and consistent with the legal authority cited in the preamble to the proposed rule (64 FR 53960 at 53964)), we have revised the language in §§ 314.610(b)(3) and 601.91(b)(3) to require that this information be included in the patient labeling.

Where the evidence of effectiveness comes from studies in animals, regulating new drug or biological products as investigational drugs presents several difficulties. These difficulties have led us to this rulemaking. The proposed rule describes our concerns with relying solely on the investigational new drug regulations (64 FR 53960 at 53963) for such approvals. There may be cases, however, when an application does not meet the criteria of this rule, and approval of the product is not feasible. Should an emergency situation arise under such circumstances, it is conceivable that the product could be used under the investigational new drug regulations.

(Comment 11) Another comment suggested that, unless "lay persons" may use the product, we prohibit advertising of drug or biological products approved on the basis of evidence of effectiveness from studies in animals. The comment further recommended stringent controls on the advertising of products that could be used by "lay persons."

Such a sweeping prohibition would likely give rise to constitutional issues regarding the regulation of commercial speech. In addition, the suggestion presents serious public health concerns. A prohibition on advertising could limit health care providers' and public health and emergency preparedness officials' awareness of the products approved under this rule. Limiting awareness of these products, which are intended to

reduce or prevent life-threatening or disabling toxicity, does not seem desirable or appropriate.

We believe that the advertising provisions in §§ 314.640 and 601.94 of this rule provide adequate protection against false or misleading advertising, and no additional requirements are needed. As discussed in the preamble to the proposed rule (64 FR 53960 at 53964), we proposed the requirements pertaining to promotional materials in order to provide for the safe and effective use of these products. These requirements, along with others, are similar to those in the accelerated approval regulations in subpart H of part 314 and in subpart E of part 601. In issuing the accelerated approval regulations, we stated that the special circumstances under which those products would be approved and the possibility that promotional materials could adversely affect the sensitive risk/benefit balance justified review of promotional materials before and after approval (57 FR 58942 at 58949). Similarly, the special circumstances of all product approvals under subpart I of part 314 and subpart H of part 601 and the possibility that promotional materials could adversely affect the even more sensitive risk/benefit balance justifies advance review of promotional materials.

We intend to review all such promotional materials under these new regulations promptly, and to notify the applicant of any identified problems as soon as possible (see also 57 FR 58942 at 58950). Also as with the accelerated approval regulations' requirements for promotional materials (§§ 314.560 and 601.46), FDA may terminate the requirements for advance submission of promotional materials under these new regulations at §§ 314.650 and 601.95 if the agency determines, on its own initiative or in response to a petition submitted by the sponsor, that the requirements are no longer necessary for safe and effective use of the product. When we remove the requirement for advance submission of promotional materials, we will continue to offer a prompt review of all voluntarily submitted promotional materials.

(Comment 12) We received some comments addressing questions posed in section VII, "Discussion," of the proposed rule. In this final rule, we have addressed comments that dealt with the rule itself. Comments that dealt with questions related to the application of this rule, rather than the requirements, will be addressed if and when we draft a guidance on this subject.

III. Legal Authority

We did not receive any comments discussing our legal authority to approve new drugs and biological products based on evidence of effectiveness from studies in animals. We have concluded, for the reasons set out in section V of the proposed rule, "Legal Authority," (64 FR 53960 at 53964), that we have the legal authority to approve new drugs and biological products based on evidence of effectiveness from studies in animals.

(Comment 13) We received a comment asserting that under the court's holding in *American Pharmaceutical Association* v. *Weinberger*, 377 F.Supp. 824 (D.C.D.C. 1974) *aff'd sub nom. American Pharmaceutical Association* v. *Mathews*, 530 F.2d 1054 (D.C. Cir. 1976) (*per curiam*), we do not have the legal authority to impose the distribution controls proposed in §§ 314.610(b) and 601.61(b) (§§ 314.610(b)(2) and 601.91(b)(2) in this final rule). The comment asked that, if we disagree with their characterization of the law, distribution controls not be applied just because a product was approved under the provisions of this rule. The comment also asked that we give examples of situations where we would impose distribution restrictions.

For a full discussion of FDA's authority to impose distribution restrictions to ensure the safe use of drug products, see the agency's proposed and final rules amending part 314 by adding subpart H on accelerated approval of new drugs for serious or life-threatening illnesses (proposed rule at 57 FR 13234, April 15, 1992; final rule at 57 FR 59842, December 11, 1992). Those rules relied on sections 501, 502, 503, 505, and 701 of the act (21 U.S.C. 351, 352, 353, 355, and 372) as authority for FDA to issue regulations to help ensure the safety and effectiveness of new drugs.

We agree with the comment that distribution controls should not be placed on a product solely because it is approved under the provisions of this rule. New §§ 314.610(b)(2) and 601.91(b)(2) authorize distribution controls—they do not require them.

We do not believe it would be useful to give examples of situations where distribution controls may be necessary to ensure safe use of the product. Products approved under this rule could be indicated for widely differing conditions, and those products could be used in unique circumstances presenting many distinct safety concerns. It would not be practical to try to devise a list of representative examples of situations where distribution controls would be appropriate.

IV. Environmental Impact

The agency has determined under 21 CFR 25.30(h) that this action is of a type that does not individually or cumulatively have a significant effect on the human environment. Therefore, neither an environmental assessment nor an environmental impact statement is required.

V. Federalism

FDA has analyzed this final rule in accordance with the principles set forth in Executive Order 13132. FDA has determined that the rule does not contain policies that have substantial direct effects on the States, on the relationship between the National Government and the States, or on the distribution of power and responsibilities among the various levels of government. Accordingly, the agency has concluded that the rule does not contain policies that have federalism implications as defined in the Executive order and, consequently, a federalism summary impact statement is not required.

VI. Analysis of Impacts

FDA has examined the impacts of the final rule under Executive Order 12866 and the Regulatory Flexibility Act (5 U.S.C. 601–612) (as amended by subtitle D of the Small Business Regulatory Fairness Act of 1996 (Public Law 104–121)) and the Unfunded Mandates Reform Act of 1995 (Public Law 104–4). Executive Order 12866 directs agencies to assess all costs and benefits of available regulatory alternatives and, when regulation is necessary, to select regulatory approaches that maximize net benefits (including potential economic, environmental, public health and safety, and other advantages; distributive impacts; and equity). Unless the agency certifies that the rule is not expected to have a significant economic impact on a substantial number of small entities, the Regulatory Flexibility Act requires agencies to analyze regulatory options that would minimize any significant economic impact of a rule on small entities. Section 202 of the Unfunded Mandates Reform Act (Public Law 104–4) requires that agencies prepare an assessment of anticipated costs and benefits before proposing any rule that may result in expenditure by State, local, and tribal governments, in the aggregate, or by the private sector, of $100 million in any one year (adjusted annually for inflation).

The agency has determined that the rule is consistent with the principles set forth in the Executive order and in these statutes. FDA finds that this rule will not have an effect on the economy that exceeds $100 million in any one year (adjusted for inflation). The current inflation-adjusted statutory threshold is about $110 million. Therefore, no further analysis is required under the Unfunded Mandates Reform Act. Because this rule does not impose any new costs on small entities, FDA certifies that this rule will not result in a significant economic impact on a substantial number of small entities. Thus, the agency need not prepare a Regulatory Flexibility Analysis. The agency reached the same conclusions in its proposed rule. FDA has not received any new information or comments that would alter its previous determinations.

VII. The Paperwork Reduction Act of 1995

This final rule contains information collection provisions that are subject to review by the Office of Management and Budget (OMB) under the Paperwork Reduction Act of 1995 (44 U.S.C. 3501–3520). The title, description, and respondent description of the information collection provisions are shown below with an estimate of the annual reporting and recordkeeping burden. Included in the estimate is the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing each collection of information.

Title: New Drug and Biological Products; Animal Efficacy Studies.

Description: FDA is amending its new drug and biological product regulations to allow appropriate studies in animals in certain cases to provide substantial evidence of effectiveness of new drug and biological products used to reduce or prevent the toxicity of chemical, biological, radiological, or nuclear substances when adequate and well-controlled efficacy studies in humans cannot be ethically conducted because the studies would involve administering a potentially lethal or permanently disabling toxic substance or organism to healthy human volunteers and field trials are not feasible prior to approval. In these circumstances, when it may be impossible to demonstrate effectiveness through adequate and well-controlled studies in humans, FDA is providing that certain new drug and biological products intended to treat or prevent serious or life-threatening conditions could be approved for marketing based on studies in animals, without the traditional efficacy studies in humans. FDA is taking this action because it recognizes the importance of improving medical response capabilities to the use of lethal or permanently disabling chemical, biological, radiological, and nuclear substances in order to protect individuals exposed to these substances.

Respondent Description: Businesses and other for-profit organizations, and nonprofit institutions.

TABLE 1.—ESTIMATED ANNUAL REPORTING BURDEN[1]

21 CFR Section	No. of Respondents	Annual Frequency per Response	Total Annual Responses	Hours per Response	Total Hours
314.610(b)(2) and 314.630 601.91(b)(2) and 601.93	1	1	1	5	5
314.610(b) and 314.640 601.91(b) and 601.94	1	1	1	240	240
Total					245

[1] There are no capital costs or operating and maintenance costs associated with this collection of information.

TABLE 2.—ESTIMATED ANNUAL DISCLOSURE/RECORDKEEPING BURDEN[1]

21 CFR Section	No. of Record-keepers	Annual Frequency per Recordkeeping	Total Annual Records	Hours per Recordkeeper	Total Hours
314.610(b)(2) and 314.630 601.91(b)(2) and 601.93	1	1	1	1	1
314.610(b) 601.91(b)	1	1	1	1	1
Total					2

[1] There are no capital costs or operating and maintenance costs with this collection of information.

FDA estimates that only one application of this nature may be submitted every 3 years; however, for calculation purposes, FDA is estimating the submission of one application annually. FDA estimates 240 hours for a manufacturer of a new drug or biological product to develop patient labeling and to submit the appropriate information and promotional labeling to FDA. At this time, FDA cannot estimate the number of postmarketing reports for adverse drug or biological experiences associated with a newly approved drug or biological product. Therefore, FDA is using one report for purposes of this information collection. These reports are required under parts 310 and 600 (21 CFR parts 310 and 600), and 314. Any burdens associated with these requirements will be reported under the adverse experience reporting (AER) information collection requirements. The estimated hours for postmarketing reports range from 1 to 5 hours based on previous estimates for AER; however FDA is estimating 5 hours for the purpose of this information collection.

The majority of the burden for developing the patient labeling is included under the reporting requirements; therefore, minimal burden is calculated for providing the guide to patients. As discussed previously, no burden can be calculated at this time for the number of AER reports that may be submitted after approval of a new drug or biologic. Therefore, the number of records that may be maintained also cannot be determined. Any burdens associated with these requirements will be reported under the AER information collection requirements. The estimated recordkeeping burden of 1 hour is based on previous estimates for the recordkeeping requirements associated with the AER system.

The information collection provisions in this final rule have been approved under OMB control number 0910–0423. This approval expires December 31, 2002. An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number.

List of Subjects

21 CFR Part 314

Administrative practice and procedure, Confidential business information, Drugs, Reporting and recordkeeping requirements.

21 CFR Part 601

Administrative practice and procedure, Biologics, Confidential business information.

Therefore, under the Federal Food, Drug, and Cosmetic Act and under authority delegated to the Commissioner of Food and Drugs, 21 CFR parts 314 and 601 are amended as follows:

PART 314—APPLICATIONS FOR FDA APPROVAL TO MARKET A NEW DRUG

1. The authority citation for 21 CFR part 314 continues to read as follows:

Authority: 21 U.S.C. 321, 331, 351, 352, 353, 355, 355a, 356, 356a, 356b, 356c, 371, 374, 379e.

2. Subpart I, consisting of §§ 314.600 through 314.650, is added to read as follows:

Subpart I—Approval of New Drugs When Human Efficacy Studies Are Not Ethical or Feasible

Sec.
314.600 Scope.
314.610 Approval based on evidence of effectiveness from studies in animals.
314.620 Withdrawal procedures.
314.630 Postmarketing safety reporting.
314.640 Promotional materials.
314.650 Termination of requirements.

Subpart I—Approval of New Drugs When Human Efficacy Studies Are Not Ethical or Feasible

§ 314.600 Scope.

This subpart applies to certain new drug products that have been studied for their safety and efficacy in ameliorating or preventing serious or life-threatening conditions caused by exposure to lethal or permanently disabling toxic biological, chemical, radiological, or nuclear substances. This subpart applies only to those new drug products for which: Definitive human efficacy studies cannot be conducted because it would be unethical to deliberately expose healthy human volunteers to a lethal or permanently disabling toxic biological, chemical, radiological, or nuclear substance; and field trials to study the product's effectiveness after an accidental or hostile exposure have not been feasible. This subpart does not apply to products that can be approved based on efficacy standards described elsewhere in FDA's regulations (e.g., accelerated approval based on surrogate markers or clinical endpoints other than survival or irreversible morbidity), nor does it address the safety evaluation for the products to which it does apply.

§ 314.610 Approval based on evidence of effectiveness from studies in animals.

(a) FDA may grant marketing approval for a new drug product for which safety has been established and for which the requirements of § 314.600 are met based on adequate and well-controlled animal studies when the results of those animal studies establish that the drug product is reasonably likely to produce clinical benefit in humans. In assessing the sufficiency of animal data, the agency may take into account other data, including human data, available to the agency. FDA will rely on the evidence from studies in animals to provide substantial evidence of the effectiveness of these products only when:

(1) There is a reasonably well-understood pathophysiological mechanism of the toxicity of the substance and its prevention or substantial reduction by the product;

(2) The effect is demonstrated in more than one animal species expected to react with a response predictive for humans, unless the effect is demonstrated in a single animal species that represents a sufficiently well-characterized animal model for predicting the response in humans;

(3) The animal study endpoint is clearly related to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity; and

(4) The data or information on the kinetics and pharmacodynamics of the product or other relevant data or information, in animals and humans, allows selection of an effective dose in humans.

(b) Approval under this subpart will be subject to three requirements:

(1) *Postmarketing studies.* The applicant must conduct postmarketing studies, such as field studies, to verify and describe the drug's clinical benefit and to assess its safety when used as indicated when such studies are feasible and ethical. Such postmarketing studies would not be feasible until an exigency arises. When such studies are feasible, the applicant must conduct such studies with due diligence. Applicants must include as part of their application a plan or approach to postmarketing study commitments in the event such studies become ethical and feasible.

(2) *Approval with restrictions to ensure safe use.* If FDA concludes that a drug product shown to be effective under this subpart can be safely used only if distribution or use is restricted, FDA will require such postmarketing restrictions as are needed to ensure safe use of the drug product, commensurate with the specific safety concerns presented by the drug product, such as:

(i) Distribution restricted to certain facilities or health care practitioners with special training or experience;

(ii) Distribution conditioned on the performance of specified medical procedures, including medical followup; and

(iii) Distribution conditioned on specified recordkeeping requirements.

(3) *Information to be provided to patient recipients.* For drug products or specific indications approved under this subpart, applicants must prepare, as part of their proposed labeling, labeling to be provided to patient recipients. The patient labeling must explain that, for ethical or feasibility reasons, the drug's approval was based on efficacy studies conducted in animals alone and must give the drug's indication(s), directions for use (dosage and administration), contraindications, a description of any reasonably foreseeable risks, adverse reactions, anticipated benefits, drug interactions, and any other relevant information required by FDA at the time of approval. The patient labeling must be available with the product to be provided to patients prior to administration or dispensing of the drug product for the use approved under this subpart, if possible.

§ 314.620 Withdrawal procedures.

(a) *Reasons to withdraw approval.* For new drugs approved under this subpart, FDA may withdraw approval, following a hearing as provided in part 15 of this chapter, as modified by this section, if:

(1) A postmarketing clinical study fails to verify clinical benefit;

(2) The applicant fails to perform the postmarketing study with due diligence;

(3) Use after marketing demonstrates that postmarketing restrictions are inadequate to ensure safe use of the drug product;

(4) The applicant fails to adhere to the postmarketing restrictions applied at the time of approval under this subpart;

(5) The promotional materials are false or misleading; or

(6) Other evidence demonstrates that the drug product is not shown to be safe or effective under its conditions of use.

(b) *Notice of opportunity for a hearing.* The Director of the Center for Drug Evaluation and Research (CDER) will give the applicant notice of an opportunity for a hearing on CDER's proposal to withdraw the approval of an application approved under this subpart. The notice, which will ordinarily be a letter, will state generally the reasons for the action and the proposed grounds for the order.

(c) *Submission of data and information.* (1) If the applicant fails to file a written request for a hearing within 15 days of receipt of the notice, the applicant waives the opportunity for a hearing.

(2) If the applicant files a timely request for a hearing, the agency will publish a notice of hearing in the **Federal Register** in accordance with §§ 12.32(e) and 15.20 of this chapter.

(3) An applicant who requests a hearing under this section must, within 30 days of receipt of the notice of opportunity for a hearing, submit the data and information upon which the applicant intends to rely at the hearing.

(d) *Separation of functions.* Separation of functions (as specified in § 10.55 of this chapter) will not apply at any point in withdrawal proceedings under this section.

(e) *Procedures for hearings.* Hearings held under this section will be conducted in accordance with the provisions of part 15 of this chapter, with the following modifications:

(1) An advisory committee duly constituted under part 14 of this chapter will be present at the hearing. The committee will be asked to review the issues involved and to provide advice and recommendations to the Commissioner of Food and Drugs.

(2) The presiding officer, the advisory committee members, up to three representatives of the applicant, and up to three representatives of CDER may question any person during or at the conclusion of the person's presentation. No other person attending the hearing may question a person making a presentation. The presiding officer may, as a matter of discretion, permit questions to be submitted to the presiding officer for response by a person making a presentation.

(f) *Judicial review.* The Commissioner of Food and Drugs' decision constitutes final agency action from which the applicant may petition for judicial review. Before requesting an order from a court for a stay of action pending review, an applicant must first submit a petition for a stay of action under § 10.35 of this chapter.

§ 314.630 Postmarketing safety reporting.

Drug products approved under this subpart are subject to the postmarketing recordkeeping and safety reporting requirements applicable to all approved drug products, as provided in §§ 314.80 and 314.81.

§ 314.640 Promotional materials.

For drug products being considered for approval under this subpart, unless otherwise informed by the agency, applicants must submit to the agency for consideration during the preapproval review period copies of all promotional materials, including promotional labeling as well as advertisements, intended for dissemination or publication within 120 days following marketing approval. After 120 days following marketing approval, unless otherwise informed by the agency, the applicant must submit promotional materials at least 30 days prior to the intended time of initial dissemination of the labeling or initial publication of the advertisement.

§ 314.650 Termination of requirements.

If FDA determines after approval under this subpart that the requirements established in §§ 314.610(b)(2), 314.620, and 314.630 are no longer necessary for the safe and effective use of a drug product, FDA will so notify the applicant. Ordinarily, for drug products approved under § 314.610, these requirements will no longer apply when FDA determines that the postmarketing study verifies and describes the drug product's clinical benefit. For drug products approved under § 314.610, the restrictions would no longer apply when FDA determines that safe use of the drug product can be ensured through appropriate labeling. FDA also retains the discretion to remove specific postapproval requirements upon review of a petition submitted by the sponsor in accordance with § 10.30 of this chapter.

PART 601—LICENSING

3. The authority citation for 21 CFR part 601 continues to read as follows:

Authority: 15 U.S.C. 1451–1561; 21 U.S.C. 321, 351, 352, 353, 355, 356b, 360, 360c–360f, 360h–360j, 371, 374, 379e, 381; 42 U.S.C. 216, 241, 262, 263, 264; sec. 122, Pub. L. 105–115, 111 Stat. 2322 (21 U.S.C. 355 note).

4. Subpart H, consisting of §§ 601.90 through 601.95, is added to read as follows:

Subpart H—Approval of Biological Products When Human Efficacy Studies Are Not Ethical or Feasible

Sec.

Subpart H—Approval of Biological Products When Human Efficacy Studies Are Not Ethical or Feasible

§ 601.90 Scope.

This subpart applies to certain biological products that have been studied for their safety and efficacy in ameliorating or preventing serious or life-threatening conditions caused by exposure to lethal or permanently disabling toxic biological, chemical, radiological, or nuclear substances. This subpart applies only to those biological products for which: Definitive human efficacy studies cannot be conducted because it would be unethical to deliberately expose healthy human volunteers to a lethal or permanently disabling toxic biological, chemical, radiological, or nuclear substance; and field trials to study the product's efficacy after an accidental or hostile exposure have not been feasible. This subpart does not apply to products that can be approved based on efficacy standards described elsewhere in FDA's regulations (e.g., accelerated approval based on surrogate markers or clinical endpoints other than survival or irreversible morbidity), nor does it address the safety evaluation for the products to which it does apply.

§ 601.91 Approval based on evidence of effectiveness from studies in animals.

(a) FDA may grant marketing approval for a biological product for which safety has been established and for which the requirements of § 601.90 are met based on adequate and well-controlled animal studies when the results of those animal studies establish that the biological product is reasonably likely to produce clinical benefit in humans. In assessing the sufficiency of animal data, the agency may take into account other data, including human data, available to the agency. FDA will rely on the evidence from studies in animals to provide substantial evidence of the effectiveness of these products only when:

(1) There is a reasonably well-understood pathophysiological mechanism of the toxicity of the

substance and its prevention or substantial reduction by the product;

(2) The effect is demonstrated in more than one animal species expected to react with a response predictive for humans, unless the effect is demonstrated in a single animal species that represents a sufficiently well-characterized animal model for predicting the response in humans;

(3) The animal study endpoint is clearly related to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity; and

(4) The data or information on the kinetics and pharmacodynamics of the product or other relevant data or information, in animals and humans, allows selection of an effective dose in humans.

(b) Approval under this subpart will be subject to three requirements:

(1) *Postmarketing studies.* The applicant must conduct postmarketing studies, such as field studies, to verify and describe the biological product's clinical benefit and to assess its safety when used as indicated when such studies are feasible and ethical. Such postmarketing studies would not be feasible until an exigency arises. When such studies are feasible, the applicant must conduct such studies with due diligence. Applicants must include as part of their application a plan or approach to postmarketing study commitments in the event such studies become ethical and feasible.

(2) *Approval with restrictions to ensure safe use.* If FDA concludes that a biological product shown to be effective under this subpart can be safely used only if distribution or use is restricted, FDA will require such postmarketing restrictions as are needed to ensure safe use of the biological product, commensurate with the specific safety concerns presented by the biological product, such as:

(i) Distribution restricted to certain facilities or health care practitioners with special training or experience;

(ii) Distribution conditioned on the performance of specified medical procedures, including medical followup; and

(iii) Distribution conditioned on specified recordkeeping requirements.

(3) *Information to be provided to patient recipients.* For biological products or specific indications approved under this subpart, applicants must prepare, as part of their proposed labeling, labeling to be provided to patient recipients. The patient labeling must explain that, for ethical or feasibility reasons, the biological product's approval was based on efficacy studies conducted in animals alone and must give the biological product's indication(s), directions for use (dosage and administration), contraindications, a description of any reasonably foreseeable risks, adverse reactions, anticipated benefits, drug interactions, and any other relevant information required by FDA at the time of approval. The patient labeling must be available with the product to be provided to patients prior to administration or dispensing of the biological product for the use approved under this subpart, if possible.

§ 601.92 Withdrawal procedures.

(a) *Reasons to withdraw approval.* For biological products approved under this subpart, FDA may withdraw approval, following a hearing as provided in part 15 of this chapter, as modified by this section, if:

(1) A postmarketing clinical study fails to verify clinical benefit;

(2) The applicant fails to perform the postmarketing study with due diligence;

(3) Use after marketing demonstrates that postmarketing restrictions are inadequate to ensure safe use of the biological product;

(4) The applicant fails to adhere to the postmarketing restrictions applied at the time of approval under this subpart;

(5) The promotional materials are false or misleading; or

(6) Other evidence demonstrates that the biological product is not shown to be safe or effective under its conditions of use.

(b) *Notice of opportunity for a hearing.* The Director of the Center for Biologics Evaluation and Research (CBER) will give the applicant notice of an opportunity for a hearing on CBER's proposal to withdraw the approval of an application approved under this subpart. The notice, which will ordinarily be a letter, will state generally the reasons for the action and the proposed grounds for the order.

(c) *Submission of data and information.* (1) If the applicant fails to file a written request for a hearing within 15 days of receipt of the notice, the applicant waives the opportunity for a hearing.

(2) If the applicant files a timely request for a hearing, the agency will publish a notice of hearing in the **Federal Register** in accordance with §§ 12.32(e) and 15.20 of this chapter.

(3) An applicant who requests a hearing under this section must, within 30 days of receipt of the notice of opportunity for a hearing, submit the data and information upon which the applicant intends to rely at the hearing.

(d) *Separation of functions.* Separation of functions (as specified in § 10.55 of this chapter) will not apply at any point in withdrawal proceedings under this section.

(e) *Procedures for hearings.* Hearings held under this section will be conducted in accordance with the provisions of part 15 of this chapter, with the following modifications:

(1) An advisory committee duly constituted under part 14 of this chapter will be present at the hearing. The committee will be asked to review the issues involved and to provide advice and recommendations to the Commissioner of Food and Drugs.

(2) The presiding officer, the advisory committee members, up to three representatives of the applicant, and up to three representatives of CBER may question any person during or at the conclusion of the person's presentation. No other person attending the hearing may question a person making a presentation. The presiding officer may, as a matter of discretion, permit questions to be submitted to the presiding officer for response by a person making a presentation.

(f) *Judicial review.* The Commissioner of Food and Drugs' decision constitutes final agency action from which the applicant may petition for judicial review. Before requesting an order from a court for a stay of action pending review, an applicant must first submit a petition for a stay of action under § 10.35 of this chapter.

§ 601.93 Postmarketing safety reporting.

Biological products approved under this subpart are subject to the postmarketing recordkeeping and safety reporting applicable to all approved biological products.

§ 601.94 Promotional materials.

For biological products being considered for approval under this subpart, unless otherwise informed by the agency, applicants must submit to the agency for consideration during the preapproval review period copies of all promotional materials, including promotional labeling as well as advertisements, intended for dissemination or publication within 120 days following marketing approval. After 120 days following marketing approval, unless otherwise informed by the agency, the applicant must submit promotional materials at least 30 days prior to the intended time of initial dissemination of the labeling or initial publication of the advertisement.

601.95 Termination of requirements.

If FDA determines after approval under this subpart that the requirements established in §§ 601.91(b)(2), 601.92, and 601.93 are no longer necessary for the safe and effective use of a biological product, FDA will so notify the applicant. Ordinarily, for biological products approved under § 601.91, these requirements will no longer apply when FDA determines that the postmarketing study verifies and describes the biological product's clinical benefit. For biological products approved under § 601.91, the restrictions would no longer apply when FDA determines that safe use of the biological product can be ensured through appropriate labeling. FDA also retains the discretion to remove specific postapproval requirements upon review of a petition submitted by the sponsor in accordance with § 10.30 of this chapter.

Dated: May 23, 2002.

Lester M. Crawford,

Deputy Commissioner.

[FR Doc. 02–13583 Filed 5–30–02; 8:45 am]

BILLING CODE 4160–01–S

DEPARTMENT OF THE TREASURY

Internal Revenue Service

26 CFR Part 1

[TD 8998]

RIN 1545–BA74

Loss Limitation Rules

AGENCY: Internal Revenue Service (IRS), Treasury.

ACTION: Temporary regulations.

SUMMARY: This document contains amendments to temporary regulations issued under sections 337(d) and 1502. The amendments clarify certain aspects of the temporary regulations relating to the deductibility of losses recognized on dispositions of subsidiary stock by members of a consolidated group. The amendments in these temporary regulations apply to corporations filing consolidated returns, both during and after the period of affiliation, and also affect purchasers of the stock of members of a consolidated group. The text of these temporary regulations also serves as the text of the proposed regulations set forth in the notice of proposed rulemaking on this subject in the Proposed Rules section in this issue of the **Federal Register**.

DATES: *Effective Date:* These regulations are effective May 31, 2002.

Applicability Date: For dates of applicability *see* § 1.337(d)–2T(g) and 1.1502–20T(i).

FOR FURTHER INFORMATION CONTACT: Sean P. Duffley (202) 622–7530 or Lola L. Johnson (202) 622–7550 (not toll-free numbers).

SUPPLEMENTARY INFORMATION:

Paperwork Reduction Act

The collection of information contained in these regulations has been previously reviewed and approved by the Office of Management and Budget under control number 1545–1774. Responses to this collection of information are voluntary. No material changes to this collection of information are made by these regulations.

An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a valid control number assigned by the Office of Management and Budget.

Books or records relating to the collection of information must be retained as long as their contents may become material in the administration of any internal revenue law. Generally, tax returns and tax return information are confidential, as required by 26 U.S.C. 6103.

Background

On March 12, 2002, the IRS and Treasury published in the **Federal Register** at 67 FR 11034 (2002–13 I.R.B. 668) temporary regulations under sections 337(d) and 1502 (the temporary regulations). The temporary regulations set forth rules that limit the deductibility of loss recognized by a consolidated group on the disposition of stock of a subsidiary member and that require certain basis reductions on the deconsolidation of stock of a subsidiary member. Section 1.1502–20T(i) of the temporary regulations provides that, in the case of a disposition or deconsolidation of a subsidiary before March 7, 2002, and for such transactions effected pursuant to a binding written contract entered into before March 7, 2002, that was in continuous effect until the disposition or deconsolidation, a consolidated group may determine the amount of allowable stock loss or basis reduction by applying § 1.1502–20 in its entirety, § 1.1502–20 without regard to the duplicated loss component of the loss disallowance rule, or § 1.337(d)-2T. For dispositions and deconsolidations that occur on or after March 7, 2002, and that are not within the scope of the binding contract rule, § 1.1502–20T(i) provides that allowable loss and basis reduction are determined under § 1.337(d)-2T, not § 1.1502–20.

Explanation of Provisions

Since the publication of the temporary regulations, several questions have been raised concerning the interpretation and application of the temporary regulations. In response to these questions, the IRS and Treasury are promulgating the regulations in this Treasury decision as temporary regulations to clarify and amend the temporary regulations as described below in this preamble. The following paragraphs describe these amendments.

Netting Rule

Commentators requested that § 1.337(d)-2T be amended to provide a netting rule similar to that set forth in § 1.1502–20(a)(4), pursuant to which gain and loss from certain dispositions of stock may be netted. This Treasury decision adds § 1.337(d)-2T(a)(4) to provide such a rule and also adds § 1.337(d)-2T(b)(4), which provides a similar netting rule for basis reductions on deconsolidations of subsidiary stock.

Time For Filing Election Described in § 1.1502–20T(i)

Section 1.1502–20T(i) currently provides that an election to determine allowable loss by applying § 1.1502–20 (without regard to the duplicated loss component of the loss disallowance rule) or § 1.337(d)–2T must be made by including a statement with or as part of the original return for the taxable year that includes the later of March 7, 2002, and the date of the disposition or deconsolidation of the stock of the subsidiary, or with or as part of an amended return filed before the date the original return for the taxable year that includes March 7, 2002, is due. Commentators noted that this provision may not permit the election to be made on an original return for the 2001 taxable year where the disposition occurs during the 2001 taxable year. The IRS and Treasury believe that it is appropriate to permit the election to be made on such a return. Therefore, this Treasury decision amends § 1.1502–20T(i) to provide that the statement may be filed with or as part of a timely filed (including any extensions) original return for any taxable year that includes any date on or before March 7, 2002. In addition, if the date of the disposition or deconsolidation of the stock of the subsidiary is after March 7, 2002, the statement may be filed with or as part of a timely filed (including any extensions) original return for the taxable year that includes such date. This latter alternative effectively permits the statement to be filed with the original return that includes the date

Appendix B

Draft Guidance for Industry: Animal Models - Essential Elements to Address Efficacy Under the Animal Rule

Guidance for Industry

Animal Models — Essential Elements to Address Efficacy Under the Animal Rule

DRAFT GUIDANCE

This guidance document is being distributed for comment purposes only.

Comments and suggestions regarding this draft document should be submitted within 60 days of publication in the *Federal Register* of the notice announcing the availability of the draft guidance. Submit comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. All comments should be identified with the docket number listed in the notice of availability that publishes in the *Federal Register*.

For questions regarding this draft document contact Rosemary Roberts, CDER, at 301-796-2210 or the Office of Communications, Training, and Manufacturers Assistance (CBER), 301-827-1800 or 800-835-4709.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

January 2009
Pharm/Tox

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Guidance for Industry

Animal Models — Essential Elements to Address Efficacy Under the Animal Rule

Office of Training and Communications
Division of Drug Information, WO51, Room 2201
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Silver Spring, MD 20993
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Food and Drug Administration
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http://www.fda.gov/cber/guidelines.htm.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

January 2009
Pharm/Tox

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Guidance for Industry[1]
Animal Models — Essential Elements to Address Efficacy Under the Animal Rule

This draft guidance, when finalized, will represent the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the FDA staff responsible for implementing this guidance. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

I. INTRODUCTION

This guidance provides information to potential sponsors (industry, academia, and government) on the development of animal models to study efficacy. The guidance focuses on the identification of the critical characteristics (essential data elements) of an animal model to be addressed when developing drug or biological products for approval or licensure, respectively, under the Animal Rule (see 21 CFR 314.600 for drugs; 21 CFR 601.90 for biological products).

This guidance does not address:

- The preclinical pharmacology/toxicology studies necessary for early drug or biological product development
- The details of study design and conduct for either animal efficacy studies or human safety studies
- The development of animal models for other purposes, such as for assessment of toxicology
- The threshold for determining that human efficacy studies are not ethical and/or not feasible

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

[1] This guidance has been prepared by the Animal Model Characterization Working Group in the Center for Drug Evaluation and Research (CDER) in cooperation with the Center for Biologics Evaluation and Research (CBER) at the Food and Drug Administration.

REFERENCES

21 CFR, Part 58. Good Laboratory Practice for Nonclinical Laboratory Studies (accessible at http://www.nal.usda.gov/awic/legislat/21cfr97.htm).

42 CFR, Parts 72 and 73. "Possession, Use and Transfer of Select Agents and Toxins," (CDC) *Federal Regist*er 70 (March 18, 2005): 13293 – 13325. (accessible at http://www.cdc.gov/od/sap/final_rule.htm)

FDA, "New Drug and Biological Drug Products; Evidence Needed to Demonstrate Effectiveness of New Drugs When Human Efficacy Studies Are Not Ethical and Feasible," *Federal Register 67* (31 May 2002): 37988-37998.

FDA, Center for Drug Evaluation and Research and Center for Biologics Evaluation and Research, guidance for industry, *Drug Interaction Studies – Study Design, Data Analysis, and Implications for Dosing and Labeling.*

FDA, Center for Drug Evaluation and Research and Center for Biologics Evaluation and Research, guidance for industry, *Drug Metabolism/Drug Interaction Studies in the Drug Development Process: Studies in Vitro.*

FDA, Center for Drug Evaluation and Research and Center for Biologics Evaluation and Research, guidance for industry, *Special Protocol Assessment.*

FDA, Center for Drug Evaluation and Research, draft guidance for industry, *Smallpox (Variola) Infection: Developing Drugs for Treatment or Prevention.*

Leffel, E.K. and Pih, L.M. (2006), Anthrax. In Swearengen, J.R. (Ed.). Biodefense: Research Methodology and Animal Models. (pp. 77-93). Boca Raton, FL: CRC Press.

USDA Animal Welfare Act, Public Law 89-544, as amended, 7 U.S.C. 2131 *et.seq.* (accessible at http://www.nal.usda.gov/awic/legislat/awa.htm).

U.S. Department of Health and Human Services, Centers for Disease Control and Prevention and National Institute of Health, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, (Washington, DC: U.S. Government Printing Office, 2007): (accessible at http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm).

WHO guidelines on nonclinical evaluation of vaccines, Geneva, World Health Organization, 2005 (WHO Technical Report Series, No. 927, Annex 1) (accessible at http://www.who.int/biologicals/publications/trs/areas/vaccines/nonclinical_evaluation/en/).

II. BACKGROUND

FDA's regulations concerning the approval of new drugs or biological products when human efficacy studies are neither ethical nor feasible are known as "the Animal Rule" (21 CFR 314.600 for drugs; 21 CFR 601.90 for biological products). The Animal Rule states that in selected circumstances, when it is neither ethical nor feasible to conduct human efficacy studies, FDA may grant marketing approval based on adequate and well-controlled animal studies when the results of those studies establish that the drug or biological product is reasonably likely to produce clinical benefit in humans. Demonstration of the product's safety in humans is still necessary (see section V.).

The purpose of this guidance is to identify the critical characteristics of an animal model that should be addressed when efficacy of the product under development will be established under the Animal Rule.

The critical characteristics discussed in section IV identify the essential elements to be considered and fully explored as part of the development of an animal model. All elements may not be achievable for each etiologic agent[2] and intervention[3] being studied. Early and frequent interactions between FDA and the sponsor are recommended to discuss these elements and any issues encountered by the sponsor. Current FDA requirements for establishing the safety of a product in humans continue to apply. Although the discussion in this guidance touches on clinical safety, it is not meant to address all requirements for assurance of human safety.

III. ANIMAL RULE CONSIDERATIONS

To develop an animal model to demonstrate efficacy, the sponsor should obtain information on the natural history of the disease or condition in both humans and animals, on the etiologic agent, and on the proposed intervention. Data from the human experience with the etiologic agent and/or with the intervention, if available, may support applicability of the animal model.

The Animal Rule states that FDA can rely on the evidence from animal studies to provide substantial evidence of effectiveness only when:

1. There is a reasonably well-understood pathophysiological mechanism of the toxicity of the (chemical, biological, radiological, or nuclear) substance and its prevention or substantial reduction by the product
2. The effect is demonstrated in more than one animal species expected to react with a response predictive for humans, unless the effect is demonstrated in a single animal

[2] For this document the terms *agent*, *threat agent*, or *etiologic agent* refer to lethal or permanently disabling toxic chemical, biological, radiological or nuclear (CBRN) substances regarding which efficacy studies in humans are neither ethical nor feasible. The term *challenge agent* refers to the CBRN material used in the animal studies.

[3] The terms *treatment* and *therapy* refer to any intervention that prevents or mitigates the toxicity of these etiologic agents.

species that represents a sufficiently well-characterized animal model[4] for predicting the response in humans

3. The animal study endpoint is clearly related to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity
4. The data or information on the (pharmaco) kinetics and pharmacodynamics of the product or other relevant data or information, in animals and humans, allows selection of an effective dose in humans

(21 CFR 314.610(a)(1)-(4); 21 CFR 601.91(a)(1)-(4))

If these criteria are met, it is reasonable to expect the effectiveness of the product in animals to be a reliable indicator of its effectiveness in humans.

The Animal Rule allows approval based on a single animal species, if the animal model is sufficiently well-characterized; however the usual expectation is that efficacy will be demonstrated in more than one species. In order to support approval based on one animal species, in general more than one efficacy study using that species should be conducted to demonstrate reproducibility of the results.

Data from animal studies to demonstrate dose-response and to support the dose selected for the animal efficacy studies are expected as is the case for traditional product development. Sponsors of products approved for other indications may be asked to provide additional nonclinical and/or clinical data to support approval/licensure of the proposed product for the indication under consideration.

If another regulatory pathway to approval (i.e., one using human data) is feasible and ethical, that pathway must be used (21 CFR 314.600 and 601.90). Although the Animal Rule allows development of products that would otherwise not have any route to approval, the rule reflects the Agency's recognition that many treatments that appeared effective in animals have not proved to be effective in humans. Consequently, developing animal models that will yield efficacy results that can be expected to be predictive for humans is challenging. The animal studies must be adequate and well-controlled (21 CFR 314.610 and 601.91), and should use the pertinent features of an adequate and well-controlled clinical study, such as a detailed protocol with randomization and adequate blinding and a statistical plan as described in 21 CFR 314.126.

Early and frequent interactions between FDA and the sponsor are recommended to discuss the applicability of the Animal Rule and specific areas of concern, as well as to enable the review of, and comment on, protocols prior to study initiation. FDA may seek Advisory Committee consultation before approval and/or early in the development process to discuss whether the concept of using certain animal data to support efficacy is reasonable.

All studies intended to support approval under the Animal Rule must be carried out under the procedures and controls outlined in FDA's Good Laboratory Practice (GLP) for Nonclinical Laboratory Studies regulations (21 CFR Part 58). FDA recognizes that conforming to GLP regulations in the conduct of studies on CBRN agents may present challenges. Such issues and

[4] A "sufficiently well-characterized animal model" is one for which the model has been adequately evaluated for its responsiveness.

their possible impact on study results and conclusions, should be discussed with the review division prior to conduct of the studies. In addition, the studies must comply with the Animal Welfare Act (7 U.S.C. 2131). For certain infectious agents, sponsors must adhere to the Select Agent Rule[5] and should comply with standards on the use of Biosafety Level (BSL) laboratory facilities.[6]

The animal efficacy studies conducted to support approval under the Animal Rule are likely to use a significant number of animals. Sponsors should submit detailed protocols (see 21 CFR 312.23(a)(6)) and provide for frequent monitoring throughout the study period. FDA strongly encourages sponsors to submit a development plan and to communicate frequently with the Agency when developing products under the Animal Rule. The protocols for the animal efficacy studies should be discussed with FDA, with sufficient time for FDA review and comment, prior to the study being conducted.

IV. DISCUSSION OF ESSENTIAL DATA ELEMENTS OF AN ANIMAL MODEL

This section provides further information on the Table, Essential Data Elements of an Animal Model, found in section VI.

A. Characteristics of CBRN Agent that Influence the Disease or Condition

Some characteristics of the specific chemical, biological, radiological, and/or nuclear (CBRN) agent that influence the disease or condition under study include: the challenge agent, pathogenic determinants, the route of exposure, and quantification of exposure.

1. The Challenge Agent

The challenge agent used in animal studies generally should be identical to the etiologic agent that causes the human disease. The purity of the challenge preparation should be documented when appropriate. If the challenge agent is different from the etiologic agent known to cause human disease, the sponsor should provide justification for the use of this challenge agent and explain why, when used in the proposed animal model, it should be considered suitable for establishing effectiveness of the intervention in humans. For example, for an animal efficacy study to support approval of a radiation countermeasure, a sponsor may not be able to predict the actual radiation exposure that would follow a nuclear detonation or the subsequent fallout. In such a case, the sponsor should provide a detailed explanation of the appropriateness of the type of radiation and dose used in the study and its relevance to the clinical situation. FDA strongly recommends that the scientific approach under consideration be discussed with FDA prior to the start of the animal studies.

[5] See Select Agent Rule (42 CFR Parts 72 & 73) available at http://www.cdc.gov/od/sap/final_rule.htm.
[6] See 5th Edition of Biosafety in Microbiological and Biomedical Laboratories (BMBL), available at http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm.

167
168 *2. Pathogenic Determinants*
169
170 It should be demonstrated that the pathogenic determinants of disease in the animal
171 model are similar to those understood for humans. Pathogenic determinants can include
172 toxin production, target organs or enzyme systems, or type of radiation. For example,
173 although mice and guinea pigs are susceptible to *Bacillus anthracis*, the pathogenesis and
174 mechanism of toxicity are different from those in humans, so that these rodent species
175 may not be appropriate efficacy models for anthrax.[7] Animal species that are not
176 susceptible to the agent, or do not demonstrate the endpoint of interest (i.e., potential for
177 mortality or major morbidity that might be reduced or prevented by sufficiently effective
178 interventions) are not suitable for the efficacy studies.
179
180 *3. Route of Exposure*
181
182 In general, the animal models developed should use a route of exposure to the challenge
183 agent that is the same as the anticipated human exposure route. This is especially
184 important for conditions for which the route of exposure is directly related to
185 pathogenesis. For example, human infection with *Yersinia pestis* through flea bite, the
186 intravenous (IV) route, or aerosol exposure results in the development of bubonic,
187 septicemic, or pneumonic plague, respectively. If a sponsor is proposing a route of
188 exposure to the etiologic agent in animals that is different from what is expected in
189 humans, adequate scientific justification should be provided. FDA strongly recommends
190 that if such an approach is being considered, it should be discussed with FDA before the
191 start of the animal studies.
192
193 *4. Quantification of Exposure*
194
195 Reliable quantification and reproducibility of the challenge dose should be demonstrated.
196 When appropriate, the sponsor should describe the scalar relationship of the animal dose
197 to that anticipated in human disease. If large differences are observed, then potential
198 implications for interpretation of comparative pathogenesis, pathophysiology, and study
199 results should be discussed with FDA. Standardization of the challenge dose may be a
200 consideration in the future to ensure robust evaluation of data in the determination of
201 effectiveness.
202
203 **B. Host Susceptibility and Response to Etiologic Agent**
204
205 The animal model chosen for development should be susceptible to the threat agent. FDA
206 recognizes there may be species differences. For example, an animal species being used to study
207 efficacy for a radiation countermeasure may require a different threshold of radiation exposure to
208 develop acute radiation syndrome, but the animal species may still be appropriate for study if the
209 resulting illness and course are similar in the animal species and humans. However, if this
210 threshold differs greatly from the human threshold, the suitability of the animal model may be

[7] Leffel, E.K. and Pitt, L.M., Anthrax. In *Biodefense: Research Methodology and Animal Models.* Swearengen, J.R. ed. Boca Raton, FL. CRC Press, 2006, 77-93.

called into question. The factor that determines differences in susceptibility to the threat agent should be described to the best extent possible (e.g., see the discussion of pyridostigmine and soman in section E.2).

The response to the etiologic agent (resulting illness or injury) manifested by the animal species exposed to that agent should be similar to the illness or injury seen in humans. For example, mustard gas typically produces extensive blistering to exposed human skin. If the animal species evaluated does not have blistering as a prominent feature of exposure to mustard gas, it is unlikely that this animal model would be acceptable to the Agency. If the sponsor believes that such a model is supportive to the study of its investigational drug, the model should be discussed with the Agency and a justification should be provided

C. Natural History of Disease: Pathophysiologic Comparability

The natural history of disease in animals and in humans should be characterized, compared, and discussed with the Agency before the sponsor initiates intervention studies in animals. In some instances, use of several different models in the same development plan can be considered. Experimental parameters may need to be modified to create a condition that more closely mimics the disease in humans. For example, variola virus causes human smallpox, and humans are the only known natural host. Nonhuman primate animal models that have been studied using variola virus as the challenge agent require a large inoculum, and often the IV route of administration is used. FDA recommends that compounds found to be active in vitro against orthopoxviruses be studied in several animal models using multiple different orthopoxviruses initially. Based on data from initial studies and availability of suitably characterized models, the next step may be to assess the appropriateness of additional study in an animal model using variola.[8] Sponsors who plan to use an animal model that involves exposure to a challenge agent that is different from the known etiologic agent in humans should discuss this with the Agency along with their planned protocols and any major differences in, or limitations of, the animal model.

When comparing the disease in animals with the disease in humans, sponsors should include time to onset of disease/condition; time course of progression of disease; and manifestations, that is, signs and symptoms (severity, progression, clinical and pathologic features, laboratory parameters, the extent of organ involvement, morbidity, and outcome of disease). A single animal model may not reflect the entire spectrum of human disease. The time to onset of disease, progression of disease, and the manifestations/outcome can be influenced by many factors, including concentration and type of etiologic agent, virulence or lethal potential of the etiologic agent, route of exposure, and other host factors including immune status.

[8] See FDA's draft guidance for industry *Smallpox (Variola) Infection: Developing Drugs for Treatment or Prevention.* Once finalized this guidance will represent the Agency's thinking on this topic.
Also, we update guidances periodically. To make sure you have the most recent version of a guidance, check the appropriate (CDER or CBER) guidance Web site, listed on the second title page of the guidance.

Contains Nonbinding Recommendations

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1. Time to Onset of Disease/Condition

The time to onset of disease/condition in animals should be reasonably similar to that in humans. Factors such as strain of the infective microorganism, route of exposure, and/or the level of exposure (i.e., concentration of the chemical, biological, radiological, or other etiologic agent) can influence time to disease/condition onset.

2. Time Course of Progression of Disease/Condition

The progression of the disease/condition in animals should be similar to that of the disease in humans to allow for observation of the effects of intervention. For example, hamsters challenged with anthrax have an extremely rapid disease progression. Thus, this species is not useful for testing the efficacy of products for the treatment of anthrax in humans. Furthermore, the clinical course of disease in the animal may be more rapid than that in the human as a result of experimental conditions, such as the route of exposure (e.g., an IV route of exposure may alter many characteristics including the time course of disease). The change in the clinical course may result in making disease recognition, intervention, and assessment of outcome more difficult. Showing the effect of an intervention may be more challenging when the time between onset of disease and death is short.

3. Manifestations (signs and symptoms)

The disease manifestations, including clinical signs and their known time course, laboratory parameters, histopathology, gross pathology, and the outcome (morbidity or mortality), should be compared between untreated animals and untreated humans (e.g., historical information). Differences should be clearly noted and explained based on the understanding of the pathophysiologic differences between the species, with due acknowledgment of the limitations that may arise where this level of understanding is limited. Because certain disease manifestations in humans (e.g., fever and shortness of breath) may be difficult to discern in animals through clinical observation, a sponsor may need to use more refined techniques, such as telemetry, to evaluate affected animals. Animals in the natural history studies as well as animals in the efficacy studies should be observed with greater frequency over the entire course of the day than would be typical of most nonclinical (pharmacology/toxicology) animal studies. This is especially true when the primary endpoint is mortality and animals are being evaluated in the context of prospectively-defined euthanasia criteria. With a mortality endpoint, animal welfare and sample integrity need to be addressed. Sample integrity (e.g., cultures, histology) may be compromised if not obtained just prior to or immediately after death or euthanasia. Study results may be influenced by the criteria used. Study personnel should be blinded to treatment and should follow observation and euthanasia criteria to minimize the possibility of unnecessary suffering of moribund animals.[9]

[9]Refer to Animal Welfare Act (7 U.S.C. 2131).

D. Trigger for Intervention

Identification of the trigger for intervention in the animal studies is critical to defining the timing of the intervention. Because animals cannot simulate the health-seeking behavior manifested by humans, the trigger for intervention should be accurately defined in the animal model. If signs and symptoms in the animal model closely resemble those in humans, these can serve as the trigger for intervention when they are recognized in the individual animal. However, in the absence of disease-defining manifestations, certain biological parameters should be used to identify the time for initiation of treatment if they are known to be relevant to the diagnosis of human disease and if a relationship to the likely diagnostic process and timing in human use of the product can be shown. For example, presence of bacteremia has been used in some efficacy studies in humans for initiation of intervention with antimicrobial drug products.[10] The utility of biological parameters/biomarkers should be demonstrated, including an analysis of the time course of the appearance of the biomarkers in animals and the onset of disease and availability of diagnostic information in humans.

When a biomarker is used as a trigger for intervention in animal studies, both the assay methodology for the biomarker and its performance characteristics should be adequately characterized. The materials and methods for the assay, as well as the raw data and results from the actual testing, should be provided for FDA review. Summary data are not sufficient. Sponsors are encouraged to initiate early discussion with FDA regarding the utility of the chosen triggers for intervention, particularly when the signs and symptoms of disease in the animal differ from those in humans.

E. Characterization of Medical Intervention

Efficacy studies should reflect the expected clinical use and indication. A particular dosage form may not be suitable for the proposed indication, so the product's dosage form should be considered in planning the development of the product. For example, an oral dosage form is preferred for postexposure prophylaxis for large populations, while an IV dosage form may be necessary for seriously ill patients. If the product is already approved for human use, there may be information on which to base the expected dose and regimen, but if there is no approved human use, the animal result will need to be translated for human use, generally requiring some PK/PD assessment. The following specific information should be submitted on the product and its characteristics in humans and in animals.

1. Product Class

The product's therapeutic class should be identified. Information that is available about other members of the class can be used to help identify potential animal models and predict/evaluate safety and efficacy issues in the proposed animal model.

[10] Refer to package insert for Cubicin, NDA No. 021572, accessible at Drugs@FDA: http://www.accessdata.fda.gov/scripts/cder/drugsatfda/.

334 *2. Mechanism of Action*
335
336 Understanding the mechanism of action may help to identify specific safety and efficacy
337 issues in the proposed animal model and to identify what additional studies should be
338 performed. The animal studies to support the approval of pyridostigmine as a
339 pretreatment for exposure to the nerve agent soman highlight the importance of
340 understanding the mechanism of action of the drug and host factors in each animal
341 species evaluated. Pretreatment with pyridostigmine was shown to decrease the lethality
342 of soman in rhesus monkeys. However, pretreatment with pyridostigmine produced
343 small and inconsistent effects on mortality in studies using rats, mice, and rabbits. The
344 effect of pyridostigmine was masked in these latter species because of high serum levels
345 of the enzyme carboxylesterase, which eliminates soman from the blood and makes these
346 species naturally highly resistant to the nerve agent. Rhesus monkeys and humans have
347 little or no carboxylesterase. To elucidate the mechanism of pyridostigmine and bridge
348 the data to the human experience, a study was conducted in rats pretreated with
349 pyridostigmine as well as a carboxylesterase inhibitor prior to exposure to soman. In this
350 study, pyridostigmine demonstrated a mortality benefit in the rats similar to that seen in
351 the rhesus monkeys.
352
353 *3. In vitro Activity*
354
355 Understanding the in vitro activity of the product will supplement known information on
356 the mechanism of action and provide early screening information.
357
358 *4. Activity in Disease/Condition of Similar Pathophysiology*
359
360 If a candidate product is targeted at a common pathway in the pathophysiologic cascade,
361 information may be available on the candidate product's use for diseases that possess a
362 similar pathway. For example, information for a product approved for the treatment of
363 neutropenia secondary to chemotherapy in cancer patients may provide useful data to
364 support studying this product for the reduction of mortality in patients with neutropenia
365 secondary to acute radiation syndrome. This information on the related condition,
366 although not required, lends further support to the candidate product's efficacy for the
367 indication to be studied.
368
369 *5. Pharmacokinetics (PK) in Unaffected Animals/Humans*
370
371 PK studies should be done in unaffected animals and humans to characterize the PK
372 profile in each and to propose dosing regimens that provide comparable drug exposures
373 in the animals and humans. Early interaction with FDA is critical to justify and establish
374 the appropriate dosing regimen for the pivotal animal studies.
375
376 *6. PK/PD (Pharmacokinetics/Pharmacodynamics) in Affected Animals/Humans*
377
378 PK information in affected animals should be compared to PK information obtained from
379 unaffected animals to establish whether the pathophysiology of a disease affects the PK

(e.g., changes in metabolic parameters may alter the pharmacokinetics). Measures of treatment response (PD measurements such as clinical outcome or exploratory biomarkers) should be proposed for discussion based on both animal studies and any available human information. If a candidate product has been used in humans for other indications, PK/PD information for the alternate indications may be supportive. It should be noted that the animal model may not predict specific disease/drug interactions. Such interactions may not be observed until the disease is treated in humans, reinforcing the critical need for postmarket clinical studies in the event of human disease.

7. PK Interactions with Medical Products Likely to Be Used Concomitantly

The absorption, distribution, metabolism, and excretion (ADME)[11, 12] of a candidate product should be studied and understood.[13] The sponsor, with knowledge of the ADME of the investigational product, should discuss with FDA other medical products that are likely to be co-administered based on the clinical scenario. Potential combinations should be considered for interaction studies that may affect the PK of either product. For example, if a candidate drug is metabolized via the cytochrome P450 system, safety or efficacy of the candidate drug could be compromised by the concomitant use of cytochrome P450 inhibitors or inducers. Such drug/drug interactions should be evaluated.

8. Synergy or Antagonism of Medical Products Likely to Be Used in Combination

Candidate products should be evaluated within the context that reflects anticipated clinical use. The sponsor, in consultation with FDA, should consider other products that are likely to be used and evaluate whether the activity of either product, when used in combination, is affected (i.e., synergy or antagonism). Examples of potential interactions include drug/drug interactions and drug/vaccine interactions. For example, it should be known whether the use of an anthrax antitoxin monoclonal will have an effect on the activity of the antimicrobials used for the treatment of disseminated anthrax disease. This potential interaction should therefore be evaluated in the animal model. This information is especially important when the therapeutic intervention is expected to include more than one medical product.

F. Design Considerations for Animal Efficacy Studies

Assessment of efficacy in animals should be robust. Adequate and well-controlled animal efficacy studies, with endpoints that demonstrate substantial clinical benefit, generally the enhancement of survival or prevention of major morbidity, are required. The time course of observation should be optimized to assess the true treatment effect. At a minimum, placebo-

[11] See *guidance for industry: Drug Metabolism/Drug Interaction Studies in the Drug Development Process: Studies In Vitro.*

[12] See *guidance for industry: Drug Interaction Studies – Study Design, Data Analysis, and Implications for Dosing and Labeling.*

[13] Biodistribution and elimination should be studied for products that are not biologically amenable to traditional ADME measures (e.g., many biologics such as vaccines, and cell and gene therapies).

controlled animal studies should be performed. If a product approved for the same indication is available, it should be used as an active comparator in addition to the investigational drug and placebo arms. The study should also be blinded to the extent feasible; any situation in which study staff might become aware of treatment assignments should be discussed with FDA in advance in view of the potential for major effects on study interpretability. Animals of both sexes should be included. FDA recognizes that there are significant supply constraints on using mature or older animals of certain animal species. The issue of the age and the immune status of the animals used in efficacy studies as compared to the intended human population should be addressed by the sponsor, when relevant. Study procedures should be uniformly applied to all study groups, and potential bias should be reduced by prespecifying the criteria for euthanasia and discussing their potential effects on interpretation of results.

Studies should be designed to mimic the clinical scenario and achieve meaningful outcomes comparable to the endpoints desired in humans. In some instances, supportive care should be administered to the animals as part of the study design. In such cases, demonstration of a product's benefit over supportive care (i.e., supportive care plus investigational drug arm should be demonstrated to be superior to the supportive care plus placebo arm) will be necessary for approval or licensure. Early discussion between the sponsor and the review division regarding the type, timing, and choice of supportive care to be administered is highly recommended.

In addition to the design characteristics already discussed in this section, the following parameters should also be addressed in the study protocols:

1. Endpoints

The product studied in the animal model should demonstrate a beneficial effect analogous to the intended outcome in humans. Primary study endpoints, which should be specifically discussed with the review division, generally are the enhancement of survival or prevention of major morbidity. The dose response for these endpoints should be explored fully and established. Although secondary endpoints can provide useful information about the animal model and the activity of the product as studied in the animal model, ordinarily, only primary endpoints can serve as the basis of approval.

2. Timing of intervention

The time to initiate intervention should support the specific indication sought for a product. If the intent is to develop the product for a treatment indication, intervention before disease is established may overestimate the effect that is likely to be seen in humans and may indeed show an effect when none would be seen in humans. A reasonable understanding of the disease course and a trigger for intervention defined by the natural history studies will be needed to design the animal efficacy studies for a treatment indication; it is important to establish the relationship of time after exposure to effectiveness. With this information, the timing for intervention can be defined, thus differentiating postexposure prophylaxis from treatment. A product to be used for postexposure prophylaxis should be administered within a reasonable window after exposure to the threat agent, but before onset of disease, with a time relationship that is

adequately justified with respect to administration of the product to humans. Proposals for pre-exposure prophylaxis should be described and discussed in advance on a case-by-case basis.

3. Route of Administration

The route of administration should reflect the indication being sought and the anticipated clinical scenario, such as mass casualty. For example, if a large number of people were exposed to anthrax, an oral dosage form would be preferred over an injectable for postexposure prophylaxis. It may be important to study multiple routes.

4. Dosing Regimen

Drugs, monoclonals, and small therapeutic proteins:

The determination of the dosing regimen should rely on sufficient PK and PD data or other relevant product information in animals and/or humans. The goals should be to (a) determine a regimen in animals that is safe and effective for the indication studied; (b) determine the corresponding exposure (i.e., AUC, Cmax) in animals that is yielded by that dosing regimen; and (c) calculate a dosing regimen in humans that will give an equivalent exposure to that seen in the animal. This will enable initial extrapolation from a dosing regimen found to be efficacious in the animal model to one expected to produce a similar benefit in humans, assuming similar exposure–response relationships. Different dosing regimens in animals and humans may be needed to provide equivalent exposure to the product and thus should be discussed with the Agency.

Vaccines:

The goal should be to develop a regimen that provides a protective immune response and that is safe. For vaccines, the dose(s) used in the animal should induce an immune response that allows for appropriate extrapolation of the animal protection data to humans based on solid scientific principles. A shorter dosing interval between inoculations as compared to the proposed clinical dosing interval may be acceptable with appropriate scientific justification.

In summary, the indication being sought drives the study design. The desired outcomes of the study (i.e., product's effect) should be determined early and carefully factored into the study design to ensure that the study meets both scientific and regulatory objectives. The Agency recommends that study protocols be prepared and submitted to FDA with enough time for FDA to review the protocols and provide feedback to the sponsor before the animal studies are initiated. The sponsor can submit these protocols (i.e., the adequate and well-controlled animal efficacy studies) with a request for review under the Special Protocol Assessment (SPA) provisions.[14]

[14] See guidance for industry: *Special Protocol Assessment.*

V. HUMAN SAFETY INFORMATION

The body of available human safety data, including data from the product's evaluation and use in other indications, is a critical component of any product's development plan and influences the risk/benefit considerations. FDA may ask for additional human safety trials to complete the safety profile of the product. Healthy human volunteers should be enlisted when there is no known significant risk in the administration of the product. If the risk is significant, study in a patient population with a similar disease should be considered if a population can be identified for which the risk/benefit balance of the study is appropriate. Sponsors should propose selection and justification of the appropriate study population in advance for FDA review and feedback.

The size of the required clinical safety database depends on many factors. Existing safety data would generally be satisfactory for products that are already marketed for another indication and known to have an acceptable safety profile in the populations that would receive the product for the new indication. When the new indication requires a longer duration of use or higher dose, additional safety data must be obtained (21 CFR 314.50(d)(5)(vi)). The type of indication being sought is another factor. For example, a product that will be used as prophylaxis in large numbers of people should have a larger safety database than a product developed for treatment of patients who are symptomatic with a disease of known high mortality. In prophylaxis scenarios, it is likely that some proportion of humans will receive the product without having been exposed to the threat agent. An adequate safety database is needed to reduce the risk of serious harm in a healthy population.

The timing and design of clinical safety studies should be coordinated with exploration of the efficacious dose and regimen in animals, in order to plan adequate studies to characterize the safety of the intended human dose, formulation, route of administration, and duration of use. Preclinical safety information should guide the choice of additional safety assessments of interest in the human safety studies. This is particularly useful for products with no prior human safety data, or when the anticipated human dosing regimen has not been previously studied or approved.

VI. ESSENTIAL DATA ELEMENTS OF AN ANIMAL MODEL

The essential data elements for the development and evaluation of animal models are listed in the table below. These elements serve as a guide. They may be modified or revised as new scientific information relevant to the condition under study becomes available. Early and frequent interactions between the sponsor and FDA are critical for feedback on proposals and appropriate discussion of uncertainties and the risk/benefit balance.

Contains Nonbinding Recommendations

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Table: Essential Data Elements of an Animal Model

DATA ELEMENTS	Animal(s)	Human
A. Characteristics of the CBRN Agent that Influence the Disease or Condition		
1. The challenge agent		
2. Pathogenic determinants		
3. Route of exposure		
4. Quantification of exposure		
B. Host Susceptibility and Response to Etiologic Agent		
C. Natural History of Disease: Pathophysiologic Comparability		
1. Time to onset of disease/condition		
2. Time course of progression of disease/condition		
3. Manifestations (signs and symptoms)		
D. Trigger for Intervention		
E. Characterization of the Medical Intervention		
1. Product class		
2. Mechanism of action		
3. In vitro activity		
4. Activity in disease/condition of similar pathophysiology		
5. PK in unaffected animals/humans		
6. PK/PD in affected animals/humans		
7. PK interactions with medical products likely to be used concomitantly		
8. Synergy or antagonism of medical products likely to be used in combination		
F. Design Considerations for Animal Efficacy Studies		
1. Endpoints		
2. Timing of intervention		
3. Route of administration		
4. Dosing regimen		
HUMAN SAFETY INFORMATION		

Contains Nonbinding Recommendations

Draft — Not for Implementation

ATTACHMENT A: ACRONYMS AND ABBREVIATIONS

ADME	Absorption, distribution, metabolism, and excretion
AUC	Area under plasma concentration-time curve from zero to infinity
BSL	Biosafety Level
CBER	Center for Biologics Evaluation and Research
CBRN	Chemical, Biological, Radiological, or Nuclear
CDER	Center for Drug Evaluation and Research
Cmax	Maximum (peak) plasma drug concentration after single dose administration
FDA	Food and Drug Administration
GLP	Good Laboratory Practices
IV	Intravenous
PD	Pharmacodynamics
PK	Pharmacokinetics
SPA	Special Protocol Assessment

Appendix C

Developing Animal Models for Use in Animal Rule Licensure: The NIAID Approach[1]

Judith A. Hewitt, Office of Biodefense Research Affairs, Division of Microbiology and Infectious Diseases, National Institutes of Allergy and Infectious Diseases; Bethesda, MD

1. INTRODUCTION

Animal models are critically important in the development of vaccines and therapeutics, not only for preliminary safety and efficacy testing to enable clinical studies in general, but in biodefense applications, they contribute the pivotal efficacy data. The gap between preliminary and pivotal animal models/studies is often judged as wide, but with the proper approach, animal models need not be the greatest hurdle to product licensure. Indeed, this perceived gap in animal models has often led to inordinate focus on this area, sometimes at the expense of other equally critical areas such as manufacturing, assays, and clinical development. Indeed, the potential for animal models to play a pivotal role is new, with the finalization of FDA's Animal Efficacy Rule (21CFR314.610 and 21CFR601.91) in May 2002. Experience with this regulatory pathway is extremely limited, leading to intense focus on the uncharted aspect: the reliance on animal models for efficacy data. The reason for the intense focus on animal models for biological threats is twofold: first, no model has yet passed the ultimate test of supporting product licensure, and second, product developers generally don't have the in-house capability to conduct animal model development in biocontainment, and researchers who do have the capability aren't directly responsible for, and sometimes only peripherally involved in, product development. NIAID's approach to animal model development is product-neutral, whereas manufacturing and clinical development are inherently product-specific activities. Product-neutral animal models are developed and assessed for their utility in testing product efficacy, and the development of the animal model is the primary outcome, even if a product is tested and information is

[1] Disclaimer: This document was not prepared by the Committee on Animal Models for Assessing Countermeasures to Bioterrorism Agents or National Research Council (NRC) staff. It was provided as background information at the request of the Committee by Judith A. Hewitt, Office of Biodefense Research Affairs, Division of Microbiology and Infectious Diseases, National Institutes of Allergy and Infectious Diseases; Bethesda, MD.

gained about that product. Ideally, NIAID uses multiple products to ensure the robustness and neutrality of the animal models. Further product specific refinements may be required, but are left to the sponsors or advanced development contracts in the context of pivotal, product-specific studies. NIAID's independent investment in the development of product-neutral animal models has been quite successful and this white paper will share examples of the most important product-neutral lessons learned and guiding principles to apply to other animal models moving forward. NIAID's approach has resulted in the development of several animal models that are now waiting on product licensure decisions as the ultimate demonstration of their utility.

Safety testing of vaccines and therapeutics typically begins in animals, where initial assessments help product sponsors and FDA understand the potential risks in humans. Data are then collected over several phases of clinical trials, with adverse event reporting as well as post-licensure monitoring contributing to our overall understanding of the safety of drugs and vaccines. As new information comes to light, changes in a product's label may be warranted, such as added warnings regarding special populations. Efficacy results are similar to safety results in that accrual of data supports continued development and use of the product.

Product licensure under the Animal Rule is only different from the usual pathway in that *all* the efficacy data comes from animal studies, by bridging data that can be obtained in both animals and humans, such as immunogenicity for vaccines and pharmacokinetics for drugs. Data that supports safety and contributes to the bridge to animal efficacy and therefore, presumably, human efficacy, must still be accrued in human subjects. FDA reviews *all* clinical trial protocols before execution, though there is no regulatory requirement for *all* animal efficacy protocols to be reviewed prior to execution. Even animal safety protocols need not be reviewed prior to execution, though executing protocols without prior review by FDA runs the risk that the data collected may not support the intended use in subsequent studies, whether human or animal.

The rigor required of any assay, animal study or clinical trial is directly determined by the decisions that will be based upon the resulting data. As a product progresses along its development pathway, increased rigor is demanded from the component assays, reagents, animal models, etc. Once each component is developed to a standard sufficient to support product licensure, further use in supporting other products of a similar nature should be straightforward.

Currently for biodefense product development against biological threats, no product is yet licensed under the Animal Rule for wide use in an event or even an emergency, therefore none of the animal models or assays has been determined to be sufficiently well developed. Indeed, the data in hand at the time of an emergency will be assessed as to their adequacy to support use of a product, therefore anticipating the nature of the emergency is the only way to potentially gauge the rigor required of the data prior to licensure; a hypothetical emergency is not the same as an actual emergency. There are two products licensed under the Animal Rule, both for chemical agents where the mechanism of action in animals and humans is extremely similar, and these products have limited licenses for use in military or first responder applications. Experience with Emergency Use Authorization for all biodefense medical countermeasures is equally limited.

Infectious disease animal models represent a dynamic system, with myriad possibilities for the relationship between host and pathogen. Both the host and the pathogen are biologic systems themselves, fraught with genetic and epigenetic variability, that when combined results in a highly dynamic situation with even more variability. An additional challenge peculiar to biodefense is that it may be difficult to relate the animal model to the human disease; we may have an incomplete picture of human disease, it may be outdated, or we may have no information at all in the case of emerging diseases. The challenge becomes even greater when using the animal model to assess the efficacy of a countermeasure—yet another player in the host-pathogen-countermeasure dynamic and yet another uncertainty in bridging efficacy in animals to humans. Will the countermeasure impact the host and

host-pathogen dynamic in a similar way in humans as in animals? This is a question scientists must wrestle.

2. DESCRIPTION OF NIAID'S ANIMAL MODEL DEVELOPMENT PROGRAM

NIAID began to address the challenge of developing biodefense medical countermeasures by first convening Blue Ribbon Panels, which developed a Strategic Plan for Biodefense Research and Research Agendas for CDC Category A Agents and Category B and C Priority Pathogens, in 2002-2003. All three documents highlighted the critical role for animal models and the needed investment. In response, NIAID's Division of Microbiology and Infectious Diseases (DMID) funded an initiative to fill some of the identified gaps in animal models, using a flexible contracting mechanism with a substantial investment. This initiative was also aided by strategic agreements with other federal agencies, such as the United States Army Medical Research Institute for Infectious Diseases (USAMRIID). The purpose of this initiative was to ensure that animal models were developed and available for product testing, in contrast to pursuing animal model development within the context of advancing specific products through product development initiatives. This program has been very successful and has therefore been renewed and extended to encompass all pathogens in the DMID portfolio.

NIAID's initial and primary focus was to develop the animal models needed for the highest priority and most advanced countermeasures: anthrax vaccines, antibiotics and antitoxins; smallpox vaccines; and plague antibiotics. Subsequent animal model development efforts expanded into other NIAID priority pathogens, as well as ensured that Project BioShield requirements would be supported by the fundamental research typically funded by NIH. NIAID participated in the development of the HHS Public Health Emergency Medical Countermeasure Enterprise (PHEMCE) Strategic and Implementation Plans, published in 2007. In 2004-05, NIAID funded animal models related to the emergence of SARS and increased emphasis on pandemic flu, especially highly pathogenic avian influenza animal models. In other words, NIAID has focused on developing infectious disease animal models in priority order and commensurate with the development of medical countermeasures.

In the immediate aftermath of 9/11 and the anthrax letters, *and* the 2002 finalization of the "FDA Animal Rule," NIAID's approach has been to ensure that countermeasure development goals are not hindered by lack of animal models and that those models meet the regulatory goals of the FDA. Generally, development and advancement of animal models is not a well-funded stand-alone area in NIH investigator-initiated research portfolio, as it is viewed as a rote activity requiring little or no innovation. Since gaps were highlighted by the Blue Ribbon Panel, NIAID sought to directly fill those gaps through contracts. NIAID's emphasis in 2003-2004 was distinctly different than the current mission of the Department of Defense's Transformational Medical Technologies Initiative, launched in 2006.

3. ISSUES AND CHALLENGES IN ANIMAL MODEL DEVELOPMENT

While the use of animal models is not new, what is new is their role in contributing critical path data, including pivotal studies, and the higher level of rigor required of those particular data. Given the dynamic nature of animal models, there are many potential challenges, and one is likely to face multiple challenges in any given program to develop a countermeasure. Anticipating and minimizing the impact of these potential challenges is important for timely progression in product development. Animal models of infectious diseases are a dynamic system involving initially just the host and the pathogen, and later including countermeasures. The actual conduct of an animal study also has an impact on its utility, not only in developing a product but informing future development efforts, referred to below as study-specific issues. This paper will first introduce these three challenges

generally (host, pathogen, study-specific issues), and then describe the practical issues experienced through NIAID's programs in Section 4, along with how they were resolved or moved forward.

3.1 Host Issues

The choice of host species is the first critical decision to be made. In many cases, the susceptible species and the nature of their disease are known from the published literature and one can begin developing models and products in a very productive manner; if not, this must first be explored. Depending on the nature of the infection, there may be subtle differences between species and understanding those differences will ultimately lead to better choices in relating the animal disease to human disease. An example that can be drawn from studies with pyridostigmine bromide (PB), given prophylactically to prevent the effects of the nerve agent Soman, relates to the susceptibility of different animal species to Soman. Species that are typically used early, such as mice and rats, demonstrated small and inconsistent effects. Further studies in guinea pigs and rhesus macaques demonstrated efficacy, but it is very desirable to test compounds in lower species first. Later studies showed that giving rats a carboxylesterase inhibitor, which increased their susceptibility to Soman and resulted in carboxylesterase levels more similar to humans, allowed the demonstration of pyridostigmine bromide efficacy. Had the carboxylesterase levels been considered up front, the use of mice and rats would not necessarily have preceded the use of guinea pigs and non-human primates; however their use has contributed to a greater understanding of Soman poisoning, the efficacy of pyridostigmine bromide and greater confidence for PB use in humans.

The choice of host species based on the infectious disease will hopefully also be a good species for the countermeasure involved, though that is not always the case. Pharmacokinetic behavior of drugs in animals is frequently different than in humans. When a protective benefit is seen in animals with lower drug exposure, it is logical that humans with a higher drug exposure would derive the same benefit. But when the lower pharmacokinetic levels in animals are only partially protective, it is difficult to know whether a more favorable pharmacokinetic profile in humans would result in better efficacy. Such differences in efficacy unfortunately would require larger studies to be conducted to demonstrate statistical benefit, and therefore careful analysis of existing data and consideration of the path forward is crucial to limiting the use of animals needlessly.

Even within a host species, there may be strain or population differences that can have an impact. One needs to consider outbred or inbred for some species, and the choices for inbred mice in particular are numerous. Without an understanding of susceptibility, it is difficult to know if the best strain has been selected. Some species are predominantly available as outbred animals, in particular, non-human primates. In that case, country of origin of the animals or the breeding stock can have an impact, as well as prior exposure to pathogens, though these impacts may not be known *a priori*. The further a program develops using a particular choice, the harder it may be to change, even if a better choice exists.

3.2 Pathogen Issues

One of the next challenges encountered in developing animal models is the challenge material itself. Selecting a strain or isolate that is representative of the pathogen is a critical decision, as it is highly desirable to use one strain/isolate throughout a product or model development program. Once a body of data is obtained using a particular strain, continued use of that strain is more likely, unless there is a good rationale for changing. Understanding variability among strains/isolates is vital to making a wise selection. If one selects the most virulent strain/isolate, then it is logical that countermeasure efficacy would extrapolate to less virulent strains/isolates; the reverse may not be true. Once a strain/isolate is

selected, it is important to understand the variables contributing to its growth and virulence, in order to control them so that the challenge material is not a variable from study to study. Ideally, one would set down a master and working bank of the pathogen and any associated cells required for growth—best practices for propagation should not be an afterthought! Assays to assess the pathogen's purity, identity and activity should also be understood and formalized as necessary to support their eventual use in quality driven studies. Genomic sequence data is particularly important for viruses, and stability programs may need to be considered for challenge material prepared in advance. While early phase studies may not require formal procedures to be established, it is advisable to adopt them as soon as possible to reduce variability. Once the strain/isolate and associated procedures are formalized, it is reasonable to also use them in discovery stage studies for future generation countermeasures. The standard challenge strain for animal studies should be included in panels for testing new candidates.

There are sometimes barriers to sharing challenge strains, beyond Select Agent regulations. Some laboratories treat a pathogen as something they own, though when it comes down to it, none outwardly claims ownership. Isolates are often considered unique, and having sole possession of the pathogen isolate makes an animal model using that isolate unique. In fact, pathogens can be propagated and perhaps that is the rationale for not sharing. NIAID firmly believes in and strives toward the sharing of strains and models and even in the replication of animal models at additional sites, as a vital component of good science. Concerns about wide distribution of strains are warranted, but distribution should not be so restricted that it prevents the development of animal models or restricts the development of countermeasures. It is important to recognize that multiple developers working on the same model serve to increase our understanding of models in a much more rapid manner than any one laboratory alone is likely to accomplish.

Having established procedures to make the challenge material, it is now time to select a challenge route and dose for testing countermeasure efficacy. There are a number of challenge routes of interest, and a number of relationships between animal model and human disease. For many diseases, the usual or expected route of transmission is known and therefore animal model develop can utilize the same route, where disease should progress similarly to humans. In some cases, the route for testing is not the natural route but rather one that has potential for biothreat use, and there may not be information on the human disease course with that route. In some cases the route of transmission is known, but replicating human disease progression in an animal model is the hurdle to be overcome. And some pathogens can be transmitted by several routes resulting in different diseases. Often the challenge route of interest is selected, either because it is known to be the natural route of infection or because it is the route anticipated in a deliberate release. If the challenge route is not known, it will need exploration.

Selecting a challenge dose requires additional information; the simplest scenario is a threshold dose required for disease, and a more complicated scenario may be encountered when a pathogen exhibits a dose response resulting in different disease patterns. Selection of a challenge dose and route may have an impact on the requirements for pathogen and therefore procedures to grow the challenge stock. Current technology for aerosol challenges delivers only a fraction of the aerosolized material into the animal(s), compared to parenteral challenge routes where the pathogen is delivered directly into the animal and one must only consider a slight overage to ensure quantitative transfer. The requirements for pathogens and concomitant changes in culture methods to accommodate challenge objectives may have unintended impacts if not carefully considered.

The impact of countermeasures on disease can similarly have a dependence on the challenge route. For newly emerging diseases, the inquiry is even greater and will rely on careful epidemiological investigations. Diseases with relatively few human cases, such as Ebola, may lead to an incomplete understanding of actual transmission, which can be further complicated by findings of

seroprevalence in the absence of disease. Of course, animal studies can also be used to help understand transmission.

Aerosol challenge technology has steadily evolved so that the challenge dose can be more precisely measured. One variable that is not as well understood is the influence of particle size on disease progression. Particle size will have an impact on deposition sites within the respiratory tract, but the impact of various deposition sites on disease progression in animal models is not fully understood. The particle size for aerosolized Bacillus anthracis spores has been well established through years of research, but our understanding is not as great when it comes to other pathogens, such as viruses and vegetative bacteria. The boundaries around particle size and deposition site for human to human transmission of smallpox are not understood, though NIAID has begun some preliminary studies to examine the effect of aerosol particle size on disease progression in rabbit/rabbitpox and cynomolgus/monkeypox models.

3.3 Study-Specific Issues

Finally, it cannot be overlooked that the conduct and reporting of specific studies can have an impact on a model and its further development. If one has a certain objective for a study, then one can only draw conclusions related to the observations made or endpoints measured within the boundaries of the quality systems applied and the intended use for the data. For example, the selection of a qualitative endpoint or assay only allows you to draw qualitative conclusions, even if an assay could be further developed to perform in a quantitative manner. The sampling or observation frequency limits the conclusions one can draw about timing or kinetics. An assay that provides pivotal information should be performed to the highest quality standard possible, and any assays performed below such a level should be acknowledged as such; in other words, it is important not to oversell assay results without building a good assay. Results reported in the scientific literature may lack sufficient detail for replication, such as methods for growth of the challenge material, critical parameters in the use of a particular assay, etc. Some of this information may be considered proprietary or have dual-use considerations, nevertheless, there is an impact on the ability of others to replicate the model if the reporting is not complete. Negative data may never be published, therefore not contributing to the scientific community's understanding of the model, even if data are mentioned but not presented in sufficient detail and perhaps leading to others to repeat studies. Anecdotal evidence and casual observations can be critical to the advancement of animal models, yet they are difficult to handle in the scientific literature and to inform future studies.

4. 4. ANIMAL MODEL DEVELOPMENT AND TESTING IN PRACTICE

What NIAID has learned over the past seven years of our animal model development program not only advances specific models, it's also translatable to other, future programs. This section will describe the NIAID's most valuable lessons learned, organized by pathogen, concluding with some pathogen-independent observations on study-specific issues encountered. The examples presented here are germane to the development of animal models and do not represent all of the work performed for NIAID; product specific information such as correlates of protection, while applicable to any vaccine, do not necessarily inform animal model development.

4.1 Anthrax

One of the first and most successful models NIAID has developed is a rabbit model of inhalational anthrax that demonstrates added benefit of post-exposure vaccination in addition to a partially

protective antibiotic regimen. The antibiotic regimen was intentionally developed to be partially protective and not to model the licensed human regimen; the licensed regimen would have been far more protective in the context of an experimental animal study. This model was developed over a two-and-a-half year period, considerably longer than originally anticipated. It took even longer to get all the development reports and though they were formally submitted to FDA over two-and-a-half years ago, the model has yet to be rigorously tested in a regulatory context as supportive of Emergency Use Authorization (EUA), product licensure, or a label indication. However, NIAID is confident in this model and considers the product-neutral development to be completed. There may be refinements required along the regulatory pathway for specific products, but we now have a strong scientific basis for understanding how the model should behave.

Could this model have been developed in less than two-and-a-half years? Yes, under certain conditions that may or may not have been possible. The original antibiotic, ciprofloxacin, was not well tolerated by the rabbits; rabbits are not a species of choice for antibiotic testing and there were not enough data to support a final, best choice of antibiotic at the outset. The second antibiotic (levofloxacin) worked well; one could estimate that approximately 3-4 months could have been saved if this had been known through prior experience or the literature. The second obstacle encountered was related to the challenge material. The time course of disease was notably different in the first proof-of-concept study combining vaccination with antibiotics. While a trend toward added benefit was seen, it was not statistically significant as animals succumbed to disease more quickly than in previous similar studies, both with and without antibiotics. The anthrax spores used in the proof-of-concept study had passed virulence testing, but the timing of the disease was different from studies using other spore lots. Studies were repeated, using spores eliciting the typical disease course, along with additional, refined antibiotic regimens based on consultation with experts in the pharmacodynamics of the antibiotic. These repeat studies were successful and consistent, quickly leading to two more studies with two additional vaccines, demonstrating robustness of the model. The setback from this second obstacle amounted to a total loss of 9 months. The relationship between the qualities of the spores and the timing of disease is not fully understood; only that it is an important consideration in the context of this model and needs to be controlled. In hindsight, the aberrant spores had the same virulence when considering an absolute measure of virulence (LD50) but not when considering time to lethality; this was seen in multiple species, including the lot release test in guinea pigs. Lastly, NIAID originally only planned to test a second vaccine for robustness, but given the availability of a third vaccine, the issues encountered, and the high priority placed on anthrax preparedness, an additional vaccine study was added for greater robustness, adding 3 more months. Beyond the two-and-a-half year model development period, NIAID elected to perform an additional study to test an assumption made in earlier studies on the appropriate time to initiate antibiotics and vaccination. This study demonstrated that our assumption had indeed been correct and could not be further refined; had NIAID empirically determined the start time in the course of model development, or made an incorrect assumption, additional time would have been required. The development time for this model was impacted both positively and negatively based on the knowledge available for rational study design. In retrospect, the obstacles encountered led to a better understanding of some of the critical parameters around this animal model.

In contrast to the robust rabbit post-exposure vaccination model, development of a similar model in non-human primates has met many difficulties. In fact, six years after beginning this model, the body of evidence suggests that it may not be possible to develop such a model without using a very large number of animals. Ironically, it was anticipated that the non-human primate model would be developed ahead of the rabbit model, as there was already a publication combining antibiotics and vaccine (Friedlander et al, 1993) and used to license antibiotics; NIAID's program began from this starting point. An obstacle was immediately encountered that set us back 2 years and 9 months before

finding an alternate path. Inhalational anthrax is considered to be highly lethal, yet a number of rhesus macaque controls survived challenge, which the literature and anecdotal evidence did not lead us to expect. Given the dynamic nature of animal models of infectious disease, there were multiple variables to consider. Initial efforts focused on the challenge spores and the aerosol delivery. It became clear that animals were being exposed to spores and resolving the infection, though doubts remained about the actual challenge dose received. Higher challenge doses were tested, to no avail. At that time, the cynomolgus macaque was beginning to gain favor as an alternate species, due to greater availability than rhesus macaques. When NIAID switched from using rhesus to cynomolgus macaques, the control survivor rate dropped from ~30% to <10%, low enough to rationally design reasonably-sized studies that could yield statistical significance. However, we quickly encountered the next obstacle: the response of non-human primates to antibiotics is strong yet variable. Further manipulation of the dose, duration and even the choice of antibiotic, still has not defined an antibiotic regimen that is consistently partially protective. NIAID now believes that the animals themselves are the source of this poorly understood variability, and that it may not be possible to control.

There is a study published on the combination of antibiotics and vaccines in a non-human primate model (rhesus) that achieved statistical significance for the added benefit of vaccination over antibiotics alone (Vietri et al, 2006). There are a couple of important differences between this study and the approach NIAID took. First, the antibiotics and vaccine were administered 1-2 hours after challenge. This most certainly does not reflect realistic capabilities and therefore may overestimate the efficacy of this combination regimen in a real-world scenario. Secondly, it uses a full human dose of vaccine, given three times. While data on the immune response were not presented, it is likely that the non-human primate immune response to this regimen exceeds that which can be achieved by humans, again potentially overestimating the value of vaccination in humans. It does, however, demonstrate that statistical benefit *can* be achieved under *these* experimental conditions. These data could be extrapolated to conclude that vaccines are capable of eliciting protective immune responses in animals that have been exposed and treated with antibiotics, under more realistic experimental conditions, even if the outcome (survival) does not allow us to differentiate the effect statistically. The 2007 Anthrax Vaccines: Bridging Correlates of Protection in Animals to Immunogenicity in Humans Workshop participants wrestled with this issue, though without the benefit of NIAID's non-human primate data set. In other words: vaccine works under certain conditions, antibiotics work under certain conditions, neither interferes with the other and can be demonstrated to help the other under suboptimal conditions, therefore even under the best or real conditions, it is likely that co-administration may help and certainly won't hurt. Indeed, physicians are likely to base their decisions not on whether an individual patient would individually experience added benefit, but whether it is reasonable and available. Basic/applied research data such as in the Vietri publication can be extremely valuable in providing context for animal models. It remains to be determined exactly what data will be required in a regulatory environment to support a post-exposure prophylaxis indication for anthrax vaccines.

In collaboration with USAMRIID, NIAID has been developing a treatment model of inhalational anthrax. USAMRIID compared the natural history of anthrax disease in rhesus macaques, cynomolgus macaques and African green monkeys, and have pursued the African green monkey model for further development. In the course of this work, some very important observations have been made, notably that animals with other infections are able to survive an aerosol challenge, presumably due to activation of innate immune function. Also, there were more rhesus macaque survivors than cynomolgus macaque survivors, in agreement with NIAID's vaccine program. In most cases, USAMRIID identified the underlying infection, but not in all cases. It is an intriguing hypothesis that control survivors may have an undetected infection which alters immune function and therefore gives the animal advantage over the pathogen. Subtle differences are also seen in the rabbit model when using rabbits from different sources, which could be due to genetic differences and/or non-symptomatic underlying

infections. This is an important area for future research, as it may impact our ability to develop models for other diseases. A possible solution is to use pathogen-free animals on all studies in order to reduce variability due to immune activation, however, that does not reflect the diversity found naturally in the human population, and specific-pathogen free animals vary by source. Indeed the impact of the microbiome on health is a new area of investigation that holds much promise for better understanding the human diseases to be modeled in animals.

The development of a therapeutic model for inhalational anthrax has benefitted from the work pursuing vaccine models. There are many common aspects of vaccine and therapeutic models, such as understanding the natural progression of disease, using the same challenge material, and understanding adjunctive therapies/regimens that might be used for both or each as an adjunct to the other. In some cases, assays and SOPs developed for one model may serve the other. One unique feature of anthrax treatment models that required additional work was the development of an assay that could be used to diagnose a diseased state, akin to the symptoms that might cause a person to seek medical attention. Bacteremia has long been an integral assay in anthrax animal studies, but the classical culture assay requires overnight growth at a minimum. PCR approaches would yield more rapid results, but suffer the disadvantage that they don't necessarily indicate the presence live organisms, rather they indicate the presence of genetic material. An electrochemiluminescent (ECL) assay was developed that detects the presence of toxin, notably the protective antigen component of toxin, as a reflection of bacteremia. This assay yields rapid results, in a matter of a few hours, thereby increasing the ability to initiate treatment in a timely fashion. The validation of this new assay for use as a treatment trigger requires a reasonable data set to demonstrate that a positive result is highly correlated with disease as assessed by other methods. Collection of such a data set is time consuming and will benefit from multiple sources of data being collected together as well as a good understanding of different assays and how they behave, or standardization of a single assay as a gold standard. The sooner these approaches are begun, the sooner the result will be achieved. It is noteworthy that the 2004 FDA Workshop on Strategies for Developing Therapeutics That Directly Target Anthrax and Its Toxins did not discuss the assay now being used. The US government had signaled a desire to purchase such products but had not yet communicated that a treatment indication was the most important aspect of the desired target product profile. USAMRIID had already developed an assay for detection purposes, yet the validation as a treatment trigger in animals had not begun and is still in progress.

Even after including the time to lethality as part of the LD_{50} spore release test, NIAID did have a treatment study that was an outlier relative to other treatment studies. We still do not fully understand this study, but there were several differences between it and other studies: the source of rabbits was different, including their specific pathogen free (SPF) status; the venous access ports malfunctioned resulting in greater handling of the animals; and the effect of spore lot cannot be ruled out.

Now that these models are nearly as far as they can go in a product-neutral fashion, the next step planned is replicating these models at additional sites, one of the final tests of a good model.

4.2 Smallpox

NIAID had very clear guidance from FDA that animal models to support licensure of a next generation smallpox vaccine would require a respiratory challenge route, not the intravenous challenge model that was the most advanced model at that time. Our approach was to compare three different respiratory challenge routes, namely intranasal, intratracheal and aerosol, by first determining the dose required to create disease most similar to human smallpox, and secondly to examine the course of disease with one dose by a pathogenesis study encompassing serial time points. It quickly became clear that this was a large amount of work and would be best accomplished across multiple sites. Therefore, in order to

reduce the variable of challenge strain, NIAID provided the actual challenge material. NIAID obtained the Monkeypox Virus Zaire 79 strain from USAMRIID because it had been used the most in intravenous studies. Another advantage to this approach was that the virus characterization could be carried out at the production site and then multiple sites could use the same material in animal studies. NIAID devised a testing scheme for identity, purity and activity testing of the virus stock. Upon testing for identity, it was discovered that the monkeypox isolate had an extremely low, but detectable, contamination with cowpox. NIAID recognized that it might not be appropriate to base critical decisions on the best challenge route using data based on a contaminated isolate, and while it was unlikely that the level of contamination (less than 10^{-6}) had a major impact on the disease manifestation, it was perceived as risky. NIAID immediately sought to obtain an uncontaminated isolate and were eventually successful. In retrospect, there were no differences seen when using a contaminated and pure stock in two small studies, but nonetheless, the risk was too great to proceed with a known contamination.

The approach of using multiple sites from the outset was new for NIAID, so we chose to replicate intravenous data across all three sites, to understand the consistency of the model in the absence of prior knowledge. NIAID also sought to harmonize procedures across the sites to the extent possible, recognizing that data collection was most important. As smallpox is not uniformly fatal and lethality was not an endpoint, a definition of severe disease was adopted by all sites. An animal was recognized as having severe disease by exhibiting one or more of the following: death or euthanasia; a poor clinical assessment score of 7 or greater on a scale of 9; or a severe rash of 100 or more pox lesions. The most important variables have been controlled and others have been harmonized to the extent practicable. There are additional variables that cannot be controlled in this program, such as the source of animals. Whether the source of animals plays a role in disease progression is unknown at this point. NIAID has studied fewer animals in monkeypox within any one route, especially the multiple respiratory routes, than with other pathogens/models, to have the same understanding of variability of these models. On the positive side, there is little reason to expect variability in the course of disease (in contrast to anthrax) and indeed animals tend to present with similar signs at similar times. There is also a very visible and incontrovertible marker of disease, namely pox lesions. This disease does demonstrate dependence on the challenge dose, and so understanding that is very important. NIAID plans to test a vaccine and a therapeutic in a monkeypox aerosol challenge model for proof-of-concept of the model. As two smallpox countermeasures have been handed off from NIAID to BARDA for further development, it is unlikely that NIAID will contribute much more data to our understanding of these models. The disadvantage of further model development occurring in the context of specific product development pathways is that such data may not become publicly available for future countermeasures. There is not a groundswell of demand for a meta-analysis of data from across studies or study sites, though NIAID would certainly participate should a meta-analysis be performed.

In the course of understanding different respiratory challenge routes, NIAID also collected detailed information on the intravenous challenge route. This route is important for antiviral countermeasures, as product developers and others in the scientific community have argued that the intravenous route of challenge, while not natural, is a more stringent test of antiviral efficacy. NIAID's studies have certainly informed the path forward for therapeutics, though the outcome remains to be seen. Earlier comments about study conduct certainly apply here. Ultimately, when all of NIAID's data is submitted to FDA, our investment in understanding disease by different challenge routes will help all smallpox countermeasures.

4.3 Plague

NIAID's biggest success in plague has been getting animal data to support a treatment indication for licensed antibiotics. This will not use the Animal Efficacy Rule, but rather 21CFR314.500, Subpart H, "Accelerated Approval of New Drugs for Serious or Life-Threatening Illnesses," as was the case for licensure of ciprofloxacin for post-exposure prophylaxis against anthrax. Subpart H allows for accelerated approval based on a surrogate endpoint, which is the serum level of antibiotics. Since licensure is based on drug levels, the assays to measure drug level must be validated, and this was our biggest hurdle. Many of the antibiotics NIAID is testing have been licensed for many years and therefore the assay technology is old and may not have been validated to today's standards. Performing pharmacokinetic studies under Good Laboratory Practices (GLP, 21 CFR 58) is a paradigm shift as well, but it's critically important to choose an antibiotic dose for animal studies that is not more favorable than that anticipated for use in humans.

As a general rule, plague is fatal and animals in a challenge study present with disease within a few hours of each other. This led to rapid development of a treatment model based on temperature as the trigger for therapy. The natural history study leading to this decision happened to have two animals that did not get a fever or become bacteremic, therefore giving great confidence in the fever trigger. Those two animals received a very low challenge dose and provided an opportunity to improve SOPs to ensure that the challenge material was not compromised. There was not a positive control for these studies, as no antibiotics were known to work in a therapeutic setting. The first antibiotic tested worked reasonably well but was not completely effective; there was no way to know if this was a limitation of the antibiotic or the animal model. Further work demonstrated that two other antibiotics were completely effective, leading to great confidence in the model. In fact, NIAID has now transferred the animal model to two additional sites where it has behaved similarly, giving us the ultimate confidence in the model overall. Getting label indications for these antibiotics is now in a regulatory arena and is beyond the scope of this paper.

4.4 Tularemia

NIAID's tularemia models are still under development; however, one observation thus far is that the methods for bacterial culture have an impact on the virulence and disease in small animal models. Vegetative bacteria are best cultured fresh, just before challenge, rather than prepared in advance and characterized by lot, as viruses and spores are typically handled. NIAID is currently working to harmonize the growth methods across various sites and models within our contracts, i.e., translating these small animal findings and methods into non-human primate studies.

4.5 General Observations on Study Conduct

This section will summarize some general lessons learned in the conduct of numerous animal studies. These are not unique to biodefense animal models, however they deserve mention regardless. NIAID aspires to develop animal models in accordance with the three R's (replace, reduce, refine), and some specific examples follow. While searching for a partially protective antibiotic regimen and encountering difficulties, NIAID took a parallel approach of using *in vitro* hollow fiber studies to determine a regimen that would be expected to limit but not completely abolish bacterial growth, as a way to achieve a partially protective regimen *in vivo*. Unfortunately, the regimen modeled in the hollow fiber studies was still too protective in animals, most likely due to immune functions not represented in the hollow fiber system. In the course of a model development program, one begins to understand the behavior of the control groups over time, as well as treatment groups. NIAID has

refined our assumptions for appropriate power calculations, and thereby reduced the number of animals in particular arms such as the control group, and weighting group sizes appropriately based on anticipated effects. NIAID has also used smaller control groups and supplemented those controls with data from historical controls; this approach works in well characterized and uniform diseases. Attention to detail in the culturing of infectious agents is vital to successful studies and using fewer animals.

One can only address questions one sets out to ask. In hindsight, NIAID performed studies which would have benefitted from the collection of additional samples at different times; on a few occasions, there were samples available to perform additional assays. Thorough development of a protocol is very important, as is careful execution and analysis of data generated. NIAID staff are notorious for plotting multiple studies on one graph and even further, multiple studies from multiple sites, multiple countermeasures, etc. While combining data sets might be considered poor practice in a statistical or regulatory setting for a therapeutic or vaccine, it is a great tool to better understand animal models.

Finally, implementing GLP is often undertaken before it is necessary, perhaps due to optimistic expectations of individual studies (or products) rather than looking at a program as a whole, including the developmental status of the animal models as well as the product under consideration. NIAID has certainly learned over time how better to determine when to conduct a study under GLP, and this is difficult to relay without knowledge of specific studies under consideration and the data set to support that study design.

5. ANIMAL MODEL DEVELOPMENT FOR BIODEFENSE ACROSS THE US GOVERNMENT

There has been a high level of interagency discussion for years, though true coordination efforts are really just now maturing. Historically, the Department of Defense had been funding the vast majority of biodefense research and product development for years, and began sharing expertise in the immediate aftermath of 9/11 and the anthrax letters. NIAID really became a major player in biodefense beginning with the 2002 budget increase. The Department of Health and Human Services similarly became a major player with the passage of Project BioShield in 2004.

Early interagency coordination efforts focused on countermeasures and reported to the Weapons of Mass Destruction Medical Countermeasures Subcommittee, originally convened by the Office of Science and Technology Policy and later chartered under the Committee on Homeland and National Security, under the National Science and Technology Council. One of the working groups chartered under this committee structure was the Product Development Tools Working Group (PDT WG), which I was asked to co-chair, charged with ensuring the availability of biocontainment facilities, animal stocks, animal model development," validated" experimental protocols and "validated" assays. This WG had representation from many agencies: DoD, DHS, HHS, FDA, NIH, and CDC. While the charge was expansive, the outputs of greatest interest focused on the status of animal models for various classes of countermeasures under consideration for acquisition. It quickly became apparent that the term "validated" meant different things to different people and that better terminology was required before moving forward. Indeed, one of the most widely used outputs of that group was the development of Technology Readiness Levels (TRLs) for product development tools such as animal models, assays and challenge material in 2006. These TRLs have been used within government for some time and are now available on the BARDA website (https://www.medicalcountermeasures.gov/TRLs_for_PDTs.aspx). Publicizing this assessment tool was delayed, in part by harmonization of DoD and HHS versions of the TRLs for countermeasures, resulting in slight adjustments in the PDT TRLs. The PDT WG performed an assessment of animal

models for the Public Health Emergency Medical Countermeasures Enterprise (PHEMCE) in early 2007, prior to finalization of the PHEMCE Implementation Plan. The PDT WG assessment confirmed that it was indeed reasonable to plan the acquisitions in the timeframes under consideration relative to the status of the product development tools. Indeed, many people lament the "lack" of animal models, but from the perspective of NIAID in 2010, the animal models have seldom been on the critical path for advancing product development. NIAID now requires animal model contractors to use this assessment tool in annual reports.

The PDT TRLs capture a few additional lessons learned by NIAID. A tool can only progress so far without concomitant investment in products, and both products and tools mature in parallel fashion. Once a tool is used to support licensure of a product, it can be used again for future products, and may or may not require refinement, depending upon the intended use of the tool and how similar new products are to products licensed using the tool. One can think of the PDT TRLs as capturing three successive phases of animal model development: product independent, product dependent and product specific. Product independent studies are the early studies of the disease itself, such as pathogenesis and natural history studies and do not require a product to be available. Product dependent studies include the proof-of-concept studies to demonstrate that an animal model can test the efficacy of a product, and therefore require a product, whether licensed or very early in development. Both product independent and product dependent studies are considered to be product neutral when considering animal model development. Product specific studies are those which are performed under GLP and in the context of testing a specific product, ideally produced under Good Manufacturing Practices (21 CFR Parts 210-211, 600-680) and using the final formulation and dose, along with assumptions about efficacy of a specific product (not a product class) for statistical power. NIAID has used products in our product-neutral animal model development program, through sources such as product-development contracts or informal partnerships, and NIAID places a strong emphasis on testing several products to ensure that the model is robust and product agnostic.

More recent efforts in interagency coordination have shifted from asking "is it possible" to "is there enough capacity" to conduct animal studies. One of these efforts is aimed toward defining the need for an expanded interagency role for testing countermeasures at USAMRIID. The other is part of the Integrated Portfolio for CBRN Medical Countermeasures, formally chartered in 2009, which is mandated to coordinate efforts between DoD and HHS. This effort makes use of many ad hoc Integrated Program Teams that contribute and consolidate information from various agencies to create one portfolio in specific countermeasure areas. Another group reporting within this structure is the Animal Studies Queue Evaluation (ASQE) Team. The ASQE Team, of which I am a co-chair, has been in existence for less than a year and has the heroic task of assessing availability of animal model capability to support all the products in a particular pipeline for a particular pathogen, and to recommend a path forward if there are constraints. Initiatives that fund countermeasure development sometimes do not take into consideration feasibility and capacity for animal studies when contracts are awarded, or perhaps only for that pathogen and not in the context of other initiatives competing for the same biocontainment space for animal studies. The US Government needs to consider this capacity and appropriate sequencing of contract awards and activities when publishing solicitations. The ASQE will strive to ensure that this is the case where animal models are concerned, but the ASQE is only a recommending body, not a decision making body.

6. FUTURE EFFORTS

NIAID has recently renewed and expanded our successful biodefense animal model contract program. The expansion represents additional models in other non-biodefense pathogens of interest to NIAID, though perhaps not of interest to this Committee. Certainly the experience and advanced level of our

biodefense animal models will inform future efforts in less mature or non-biodefense animal models. It is important that future models be developed and studies performed in the most appropriate facilities—early proof of concept models may not be the best utilization of resources if performed in the expensive and limited environment of a GLP facility. It would be highly appropriate to develop animal models in research facilities and successfully transfer them to a fully compliant GLP environment, and a history of smooth transition from research to GLP facilities will help establish a greater level of comfort in the appropriate placement of animal studies by the product development community. Research laboratories need to consider early choices that may impact the transferability of models, such as the creation of master and working banks of pathogens and standardization and definition of limits of procedures, in order to successfully transfer models to a GLP environment. The term "GLP-like" is often derided as meaningless, but it can be difficult to transfer a model from research laboratory practices to GLP without going through an intermediate of good laboratory habits and documentation, with an eye toward the ultimate goal. The current state of animal model development is progressive in nature, approached by incremental advances rather than cumulative advancements all in one study. Other approaches may be feasible, but are uncharted regarding the final goal of successful implementation in regulatory decisions.

7. SUMMARY

Over the past seven years, NIAID has developed an extensive program devoted to animal models that are coordinated with, but not direct results of, product development efforts. The product-neutral nature of NIAID's animal model program has focused on developing the best possible animal models, without the potentially competing interest of furthering a product. This approach has been very productive and has tremendously helped the regulatory framework for assessing product efficacy. NIAID's models are assessed as models in their own right, without a concomitant assessment of a product. NIAID's objective approach to animal model development, along with a high level of investment, has been very successful and should be viewed as the standard when approaching Animal Rule efficacy to support medical countermeasures for biological threats.

"*Nothing happens quite by chance. It's a question of accretion of information and experience.*"

Jonas Salk

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Appendix D

Presentations to the Committee

Animal Models for Assessing Countermeasures to Bioterrorism Agents
September 17-18 2009

Thursday, September 17, 2009	
1:00 – 2:00 pm	TMTI: Mr. Jean Reed, Deputy Assistant to the Secretary of Defense for Chemical and Biological Defense/Chemical Characterization (DATSD (CBD/CD)) and Dr. Richard Jaffe, Senior Medical Advisor, ANSER, INC *Introduction and sponsor's needs.*
2:00 – 3:00 pm	TMTI: Dr. Randall Kincaid, Scientific Director *The scientific needs and purpose of TMTI; scientific portfolio and animal models.*
3:00 – 4:00 pm	TMTI: Dr. Heather Wargo *MCM portfolio and current animal model use.*
4:00 – 5:00 pm	BARDA: Dr. Thomas Dreier *Advanced/integrated developer's needs for animal models.*
Friday, September 18, 2009	
8:00 - 10:00 am	NIAID: Dr. Michael Kurilla, Director, Office of BioDefense Research Affairs *The role of NIAID.*
12:25 – 1:30 pm	NIAID: Dr. Judith Hewitt, Chief, Biodefense Research Resources Section *Animal models and the NIAID portfolio.*

Animal Models for Assessing Countermeasures to Bioterrorism Agents
November 15-17, 2009

Sunday, November 15, 2009	
1:30 – 2:30pm	Dr. Louise Pitt, Director, Center for Aerobiological Sciences, USAMRIID *GLP studies in biocontainment: Toward Animal Rule licensure_Issues, challenges and humane endpoints.*
2:30 – 3:30pm	Dr. Thomas Hartung, Director, Center for Alternatives to Animal Testing, Johns Hopkins *Definition and validation of alternative models.*
Monday, November 16, 2009	
9:00 – 10:00am	Dr. Kenneth Drake, CEO, Seralogix *In silico approaches to disease modeling.*
10:00 - 11:00am	Dr. Rui-Ru Ji, Bristol-Myers Squibb *Transcriptional dose-response profiling.*
11:15am - 12:15pm	Dr. Lisa Hensley, Chief, Viral Therapeutics, Virology Division, USAMRIID *Telemetry uses in BSL facilities and clinical trials.*
12:45 – 2:15pm	Mr. Robert Brockway, Director, Product Marketing, Data Sciences International *Advanced telemetry methods in the context of Animal Rule.* *Respiration and safety pharmacology translational models.* Dr. Russell Bialecki, Director, Safety Pharmacology North America, AstraZeneca Pharmaceuticals *Automated PK/PD and metabolic analysis integration with chronic telemetric monitoring in rodent models.*
2:15 – 3:15pm	Dr. Steven Opal, Director, Infectious Diseases Division, Memorial Hospital of Rhode Island *Surrogate markers in translational research (where animal to human translation is not an option).*
3:15 – 4:15pm	Dr. Donald Low, Microbiologist-in-Chief, Mount Sinai Hospital, Toronto, Canada *Surrogate markers as decision-making tools in clinical medicine.*
4:30 – 5:30pm	Dr. Charles Lin, Wellman Center for Photomedicine, Massachusetts General Hospital *Non-biocontainmnet imaging in the context of Animal Rule requirements.*
5:30 – 6:30pm	Dr. Peter Jahrling, Director, NIAID Integrated Research Facility and Dr. Daniel Mollura, Staff Clinician, NIH Clinical Center, and Staff Scientist, NIAID Integrated Research Facility

	Medical imaging in biocontainment in the context of Animal Rule requirements.
	Tuesday November 17, 2009
9:00 - 10:00am	Dr. Bruce Aronow, Scientific Director, Center for Computational medicine, University of Cincinnati College of Medicine *Virulence networks' detection and host defense interaction.*
10:00 - 11:00am	Dr. Steve Niemi, Director, Center for Comparative Medicine, Massachusetts General Hospital *The animal as patient: The ICU approach to animal research subjects.*

Animal Models for Assessing Countermeasures to Bioterrorism Agents
February 3-5, 2010

Wednesday, February 3, 2010	
9:30 – 11:00 am	Dr. Steven Leary, Chair, AVMA Panel on Euthanasia *Ethical considerations of euthanasia in biodefense research.* Dr. Steve Niemi, Director, Center for Comparative Medicine, Massachusetts General Hospital *Humane endpoints in infectious diseases and biodefense research.*
11:00 am – 12:00 pm	Dr. James Roth, Director, Center for Food Security and Public Health, Iowa State University *Development of biological countermeasures for CBRN agents in animals: Lessons learned.*
2:00 – 3:00 pm	Dr. Nicholas Vietri, USAMRIID *Fine-tuning animal models under the Animal Rule.*
3:00 – 4:00 pm	Dr. Drusilla Burns, FDA *A conversation about the Animal Rule.*
4:00 – 5:00 pm	Ms. Hilde Boone, European Medicines Agency *The European perspective on early approval mechanisms for new drugs.*
Thursday, February 4, 2010	
9:00 – 10:00 am	Dr. William Smith, Acting Deputy Commander, USAMRICD *Development and licensure of pyridostigmine bromide under the Animal Rule.* Dr. Renae L. Malek, Senior Scientist, Medical Identification and Treatment Systems *Advanced developer Animal Rule perspective.*
10:00 am - 12:00 pm	Dr. Sally Bolmer, SVP, Human Genome Sciences *Insights from development of an MCM for anthrax under the Animal Rule.*
2:15 – 3:15 pm	Dr. Andrew Rowan, EVP, The Humane Society of the United States *Biodefense research and animal experimentation.*

Appendix E

Statement of Task
Animal Models for Assessing Countermeasures to Bioterrorism Agents

Summary

The National Academies will convene an ad hoc committee to examine the utility and relevance of animal models to Transformational Medical Technologies Initiative (TMTI)-funded research. The report will: 1) Evaluate how well the existing TMTI-employed or candidate animal models reflect human disease as related to the agents of interest; 2) Address the process and/or feasibility of developing new animal models for critical biodefense research, placing emphasis on the need for a robust and expeditious validation process in terms of the U.S. Food and Drug Administration's (FDA's) Animal Rule; 3) Evaluate alternatives to the use of animal models based on the premise of The Three Rs vis-à-vis the Animal Rule and FDA licensure. The evaluation will also consider the development of more humane models for infectious diseases research that do not incorporate death as an endpoint (i.e., humane endpoints).

Policy Context

A major component of the U.S. Department of Defense (DoD) efforts in biodefense is the Transformational Medical Technologies Initiative (TMTI), the goal of which is to protect warfighters from disease and biological warfare agents. Specifically, the rationale of the Initiative is to fully exploit advanced science and technology innovation in order to successfully counter future genetically engineered biological weapons and naturally emerging infectious diseases that can impact the warfighter.

In the past DoD has had a significant focus on the production of individual vaccines for diseases such as anthrax, smallpox, and plague. TMTI seeks to expand that focus to facilitate basic and applied research that will lead to the development of broad-spectrum countermeasures (preventative, prophylactic and therapeutic) that could provide multivalent solutions (for example, one drug that would offer protection from multiple types of pathogens) against advanced bio-terror threats. TMTI is

therefore funding basic and applied research designed to advance the development of such countermeasures.

There are several challenges to developing such countermeasures under each of the TMTI's Current Thrust Areas (i.e., host immune enhancement; genomic identification; nucleotide therapeutics; protein based therapeutics/biologics; small molecule/drugs; metabolomics). One area of particular concern is the need to develop countermeasures for diseases that are not endemic in the United States or in other developed countries and for which no reliable treatment exists. Further, countermeasures are called for to deal with unnatural diseases resulting from bioterrorism or biowarfare. Another potential concern with countermeasures-related research is that it is conceivable that some of its results could be used by terrorists to advance offensive biowarfare.

According to Department of Defense Instruction Number 6200.02 of February 27, 2008[1] , "personnel carrying out military operations shall be provided the best possible medical countermeasures to chemical, biological, or radiological warfare or terrorism and other health threats. The DoD Components shall make preferential use of products approved by the Food and Drug Administration (FDA) for general commercial marketing, when available, to provide the needed medical countermeasure." Therefore high priority is given to work that will facilitate FDA approval of new countermeasures developed through TMTI. Lack of scientific expertise and available information necessitates that such countermeasures research is based on experimental animal models.

Ethical constraints preclude the use of human participants in efficacy studies that could kill or permanently disable healthy human volunteers. In order to overcome this predicament, FDA promulgated the Animal Rule (21 CFR Parts 314 and 601). It is expected that many TMTI-developed products would be submitted to FDA and subject to evaluation under the Animal Rule.

Technical Context

Biomedical research depends on the use of animals in order to understand how human and non-human organisms function. Investigators use animals to understand the continuum between basic mechanisms in a single cell (e.g., enzymatic properties, gene influences) and the health and disease of the whole organism. In fact, the use of animals as working representations for a variety of human conditions offers an alternative to the use of human participants. However, in order for these models to be useful correlates, they should be reproducible and verifiable (i.e., offer proof of concept) and reliably predict the safety and efficacy of clinical trials. Animal models as surrogates for humans have mixed success. In some cases, the animals correctly model the processes occurring in humans; in others, there are similarities between the animal models and humans, but the two are not exact. Furthermore, when modeling an unknown or minimally understood process, it is difficult at the outset to determine which animal model would best approximate the human situation.

In the case of the Animal Rule, FDA allows the substitution of appropriate studies in animals as "evidence of the effectiveness of new drugs or biologicals when adequate or well-controlled clinical studies in humans cannot be ethically conducted" (the Animal Rule; 21 CFR Parts 314 and 601). Licensing the medical countermeasures mandated by TMTI under the Animal Rule is challenging due to a number of concerns. FDA approval under the Animal Rule requires validated animal models that predict the efficacy of new drugs or biologicals in humans. This relatively new approach to attaining full licensure for drugs and biologicals presents new and unique challenges such as establishing validated animal models that meet FDA requirements, working in compliance with Good Laboratory Practice (GLP) regulations in a high-containment environment, and meeting the obligation to continuously identify refinements in the field of infectious disease research in general.

[1] Department of Defense Instruction Number 6200.02; www.dtic.mil/whs/directives/corres/pdf/620002p.pdf

As stated above, it would be unethical to test these agents in humans; therefore it is essential to choose the correct animal model for investigating countermeasures to bioterrorism agents. In some cases, there may be enough information about the pathophysiology of the agent and the intervention to enable a quick and accurate determination of the appropriate model. In other cases, it may be necessary to glean information from multiple models or to use other, newly emerging non-animal methods to establish a baseline understanding of the agent(s) involved. Such new methods are coming into greater use as their applications to the study of drug efficacy and drug and chemical toxicity are being delineated. In this project, it will be necessary for the committee to consider these and other possibilities for the study of countermeasures to bioterrorism agents in making recommendations to the Department of Defense.

Statement of Task

A major component of the U.S. Department of Defense (DoD) efforts in biodefense is the Transformational Medical Technologies Initiative (TMTI), the goal of which is to protect warfighters from disease and biological warfare agents. Specifically, the rationale of the Initiative is to fully exploit advanced science and technology innovation in order to successfully counter future genetically engineered biological weapons and naturally emerging infectious diseases that can impact the warfighter.

Ethical constraints preclude the use of human participants in efficacy studies that could kill or permanently disable healthy human volunteers. In order to overcome this predicament, the U.S. Food and Drug Administration (FDA) instigated the Animal Rule (21 CFR Parts 314 and 601). It is expected that many TMTI-developed products would be submitted to FDA and subject to evaluation under the Animal Rule.

The National Academies will convene an ad hoc committee to examine the utility and relevance of animal models to TMTI-funded research and prepare a consensus report. Specifically, the committee's report will:

1. Evaluate how well the existing TMTI-employed or candidate animal models reflect the pathophysiology, clinical picture and treatment of human disease as related to the agents of interest.
2. Address the process and/or feasibility of developing new animal models for critical biodefense research, placing emphasis on the need for a robust and expeditious validation process in terms of FDA's Animal Rule.
3. Evaluate alternatives to the use of animal models based on the premise of The Three Rs (i.e., refinement, reduction, and replacement of animal use; such venues would include but not be limited to in vitro work, computational modeling, new biotechnological tools, surrogate diseases, etc.) vis-à-vis the Animal Rule and FDA licensure. The evaluation will also consider the development of more humane models for infectious diseases research that do not incorporate death as an endpoint (i.e., humane endpoints).

Appendix F

About the Authors

Committee Members

George W. Korch, Jr., PhD, (*Co-Chair*), is Senior Science Advisor to the Assistant Secretary for Preparedness and Response, Health and Human Services, and Visiting Professor, Department of Molecular Microbiology and Immunology, The Johns Hopkins Bloomberg School of Public Health. Dr. Korch retired from the U.S. Army Medical Department in 2008, where he had served in a number of leadership roles, including the Commander of the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) and the Director of the Department of Defense Medical Chemical and Biological Defense Research Program. He also served as one of the first Directors of the National Biodefense Analysis and Countermeasure Center (NBACC), Department of Homeland Security. His area of expertise is in viral and rickettsial zoonotic diseases and in medical countermeasure development (vaccines, therapeutics and diagnostics) for biodefense needs. He is the co-chair of the Federal Experts Security Advisory Panel for the National Security Staff and participates on a variety of working groups within the federal government concerning national and international biosecurity and health. He serves or has served on such committees as the Institute of Medicine's Forum on Microbial Threats, was appointed by the Maryland Governor to the State's Life Sciences Advisory Board and serves on the Standards Development Committee for the American Type Cell Culture. He has co-authored one book on medical defense against biological threats as well as published articles regarding arboviral diseases and biodefense issues.

Steven M. Niemi, **DVM,** (*Co-Chair*), is Director of the Center for Comparative Medicine at Massachusetts General Hospital and an Instructor in Pathology at Harvard Medical School. Dr. Niemi is a Diplomate and President-elect of the American College of Laboratory Animal Medicine. He is an Ad hoc Consultant with AAALAC, International, a past President of the Scientists Center for Animal Welfare, and a member of the American Association for Laboratory Animal Science, American

Veterinary Medical Association, Veterinary Cancer Society, and ILAR Council. He has published on alternatives to animal experimentation and his current research interests include alleviating distress in laboratory animals.

Nicholas H. Bergman, PhD, is Senior Principal Investigator, National Biodefense Analysis and Countermeasures Center. His research in the past has focused on the identification of targets for therapeutic intervention using genomics and proteomics, and more recently on the ways these approaches can be best used for microbial forensics. His lab includes both experimental and computational biologists, and their work has involved the use of a range of systems-level approaches in understanding the biology and pathogenesis of a variety of bacterial pathogens, including *B. anthracis*, *F. tularensis*, *S. aureus*, *S. pyogenes*, and *A. baumannii.* Dr. Bergman is a member of the Interoperability Working Group, NIH/NIAID Biodefense Proteomics Research Centers, the NIAID Systems Biology of Infectious Disease Scientific Working Group, and is the chairperson of the NBACC Institutional Biosafety Committee. He was formerly Co-Director, NIAID/University of Michigan Biodefense Proteomics Research Center. He also served as an external reviewer for the U.S. Department of State, Russian Nonproliferation Office and International Science and Technology Center; the US Department of Homeland Security/National Biodefense Analysis and Countermeasures Center (NBACC); Defence Research and Development Canada—Centre for Security Science; and U.S. Army Medical Research and Materiel Command Grants Office.

Daniel J. Carucci, MD, MSc, PhD, is the President of Global Health Consulting, Inc., providing profit and non-profit organizations, international NGOs, multilateral and service organizations who are working toward improving the impact of global health investments. The organization provides strategic and technical assistance in global health to major international private sector corporations focused on increasing demand for quality health products and services, improving integration of health services across sectors, leveraging corporate capabilities applied to global health, and supporting large-scale global health programs. As Vice President for Global Health at the United Nations Foundation, Dr Carucci supported programs and fostered diverse partnerships to address the United Nations Millennium Development Goals. He spearheaded programs to amplify the voices of African leaders for improved health in Africa, worked to increase the awareness and impact of global health investments and supported innovations in global health technology, communications and finance. As Director of the Grand Challenges in Global Health Initiative at the Foundation for the National Institutes of Health, he oversaw a $200 million investment portfolio of research programs supported by the Bill & Melinda Gates Foundation in cutting edge technologies directed at solving technical barriers to improved global health. He completed 20 years active service as a U.S. Navy physician and research scientist. As Director of the U.S. Navy Malaria Vaccine Program he led a team of scientists and physicians in cutting edge genomic approaches to the development and testing of malaria vaccines, establishing partnerships with the biotechnology industry and building clinical trial capabilities in the developing world. Dr Carucci received a Medical Degree from the University of Virginia, School of Medicine; a Masters of Science in Clinical Tropical Medicine and a Doctor of Philosophy from the London School of Hygiene & Tropical Medicine. He is an Honorary Professor of the London School of Tropical Medicine & Hygiene and the recipient of the prestigious 2002 American Medical Association Nathan Davis Award for Outstanding Government Service, the 2000 Chairman of the Joint Chiefs of Staff Award for Excellence in Military Medicine, and the1989 Operational Flight Surgeon of the Year. He has published over 70 peer-reviewed articles and book chapters. His personal awards while with the U.S. Navy include the Legion of Merit with gold star (in lieu of a second award), Meritorious Service Medal with gold star (in lieu of a second award), Navy Commendation Medal and Navy Achievement Medal.

Susan A. Ehrlich, JD, LLM retired after serving eighteen years as a judge on the Arizona Court of Appeals. She received her BA degree from Wellesley College and her JD and LLM (biotechnology and genomics) degrees from Arizona State University. Prior to joining the Arizona Court of Appeals, she was an Assistant U.S. Attorney for the District of Arizona, a Department of Justice Civil Division Appellate Section attorney, and the law clerk for the Chief Justice of the Arizona Supreme Court. She has received numerous awards throughout her career. Judge Ehrlich currently serves on the National Science Advisory Board for Biosecurity and as an adjunct professor, Department of Microbiology and Immunology, University of Texas Medical Branch – Galveston/Galveston National Laboratory.

Gigi Kwik Gronvall, PhD, is a Senior Associate at the Center for Biosecurity of UPMC and an Assistant Professor of Medicine at the University of Pittsburgh. She is an immunologist by training. She serves on the American Association for the Advancement of Science (AAAS) Committee on Scientific Freedom and Responsibility, and she participated in the European Union Visitors Programme for 2011. Dr. Gronvall served as the Science Advisor of the Commission on the Prevention of Weapons of Mass Destruction Proliferation and Terrorism from April 2009 until the Commission ended in February 2010. She has testified before Congress about the safety and security of high-containment biological laboratories in the United States and served on several task forces related to laboratory security, including a 2008 Defense Science Board task force and a 2008 National Academy of Sciences (NAS) panel charged with providing technical input on the risk of operating Boston University's National Emerging Infectious Diseases Laboratory (NEIDL). Dr. Gronvall has investigated and presented policy recommendations on the governance of science to the Biological Weapons Convention (BWC) in Geneva, Switzerland (2003, 2005, and 2006). Dr. Gronvall is an Associate Editor of the quarterly journal Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science.

Thomas Hartung, MD, PhD, is Director of the Center for Alternatives to Animal Testing (CAAT) and the inaugural Chair for Evidence-Based Toxicology in the Department of Environmental Health Sciences at the Johns Hopkins Bloomberg School of Public Health. He holds a joint appointment for Molecular Microbiology and Immunology. Dr. Hartung headed the European Centre for Alternative Methods (ECVAM) at the European Commission Joint Research Centre in Italy. Dr. Hartung joined the faculty at University of Konstanz in 1994, where served as an Assistant Professor of Biochemical Pharmacology, then as an Associate Professor in the Department of Pharmacology and Toxicology until 2002, with a focus on immunomodulatory treatments of infectious diseases and immune recognition of bacterial toxins. He has been a full professor of Pharmacology and Toxicology at Konstanz since 2003. Dr. Hartung also served as the CEO of the Steinbeis Technology Transfer Center for In Vitro Pharmacology and Toxicology (InPuT). He has authored more than 350 papers.

Elizabeth Heitman, **PhD,** is Associate Professor and Director of Clinical and Research Ethics in the Center for Biomedical Ethics and Society at Vanderbilt University Medical Center. Her primary research addresses the evaluation of education in the responsible conduct of research, and the cultural awareness and professional socialization of students and researchers. Dr. Heitman is the Director of a five-year, research ethics education program for Costa Rican biomedical researchers and research ethics review committees, sponsored by the NIH's Fogarty International Center, and Chair-Elect of the Clinical Research Ethics Key Function Committee of the Clinical and Translational Science Award (CTSA) Consortium. She is the coauthor of *The Ethical Dimensions of the Biological and Health Sciences* (with Drs. Ruth Ellen Bulger and Stanley Joel Reiser).

Malak Kotb, PhD is a Senior Research Career Scientists at the VA System, Director of the Midsouth Center for Emerging Infectious Diseases (MI-CEID), and Chairperson for the University of Cincinnati College of Medicine, Department of Molecular Genetics, Biochemistry and Microbiology. Dr. Kotb has been involved in studies of the mechanism and complex genetics of human diseases and has developed small animal models for systems genetics and systems biology of infectious diseases. Kotb established and directed the Immunology and Immunogenetics program at UTHSC and was later appointed as Director of Translational Research Programs and of the Biodefense Research Program. She was also named A.C. Mullins Endowed Professor of Translational Research and Director of the MidSouth Center for Biodefense and Security. She served on a seven member external panel for the CDC on Anthrax Vaccine, was appointed to the Advisory Board of the National Council for Preparedness and Security and was appointed to the Task Force for Preparation for Avian Flu Pandemic by the Governor of Tennessee. Dr. Kotb chaired the NIH Immunological Sciences (Host Defense and Innate Immunity) and the Immunity and Host Defense (IHD) study sections. She served on advisory boards and NIH delegations to European countries. Kotb also chaired several Ad hoc NIAID Infectious Diseases review panels. Her research has been supported by funds mainly from the VA, NIH and DOD. She has published over 175 original articles, edited two books, contributed 20 book chapters and 13 invited reviews. She continues her research activities in the fields of translation medicine, focusing on systems approaches to infectious diseases and cancer therapeutics.

Jens H. Kuhn, MD, PhD, PhD, MS, is a Managing Consultant at Tunnell Consulting, Inc., King of Prussia, PA, and Lead Virologist (Contractor) at NIH/NIAID's new maximum-containment facility, the Integrated Research Facility at Fort Detrick (IRF-Frederick) in Frederick, MD. Kuhn specializes in highly virulent viral pathogens and recently published *Filoviruses - A Compendium of 40 Years of Epidemiological, Clinical, and Laboratory Studies* (Vienna: Springer, 2008). He has studied and worked, among other countries, in Germany, Russia, South Africa, and South Korea. In the US, he rotated through the Arthropod-borne Infectious Disease Laboratory (AIDL), Ft. Collins, CO, the Centers for Disease Control and Prevention (CDC), Atlanta, GA, and the United States Army Medical Research Institute of Infectious Diseases (USAMRIID), Ft. Detrick. Frederick, MD. In 2001, Kuhn was the first Western scientist with permission to work in the former Soviet biological warfare facility "Vektor" in Siberia, Russia, within the US DoD's Cooperative Threat Reduction Program. Kuhn was a contributor to the Center for International and Security Studies at Maryland's Controlling Dangerous Pathogens Project and a member of the Center for Arms Control and Nonproliferation's CBW Scientist Working Group in Washington, DC, is a member of the editorial board of *Applied Biosafety – Journal of the American Biological Safety Association* and *Archives of Virology*, and a member of the ICTV *Filoviridae* Study Group.

C. Rick Lyons, MD, PhD, was named Director of the Infectious Disease Research Center at Colorado State University in 2010. Dr. Lyons is a physician-scientist trained as a Hematologist/Oncologist. He received his MD and doctorate from University of Texas Southwestern Medical School in Dallas, Texas. He received his doctorate in Immunology and his training in Hematology/Oncology at the Brigham and Women's Hospital in Boston, Massachusetts. He comes to Colorado State University from the University of New Mexico Health Science Center in Albuquerque where he was professor of Medicine and Director of the Center for Infectious Diseases and Immunology. His scientific expertise is in developing animal models of human diseases that can be used to translate products into humans. Dr. Lyons has over twenty five years experience in developing and performing research in animal models of infectious disease. There are three main emphases in his research: 1) Develop the most accurate animal models of infection that mimic human disease; 2) Apply cutting edge technology to analyze the endpoints during *in vivo* infection; and 3) Develop strong collaborations with internal and external

investigators to bring the most expertise to bear on these issues. In the last ten years he has focused his research on a variety of emerging infections particularly in the field of bioweapons including *Bacillus anthracis* and *Francisella tularensis* using a variety of species to examine their pathogenesis including mice, rats, rabbits and primates.

Stephen S. Morse, PhD, is Professor of Clinical Epidemiology at Columbia University's Mailman School of Public Health, co-Director of the PREDICT project of the USAID Emerging Pandemic Threats (EPT) program, and Visiting Professor at the University of California, Davis. He was also founding director of the Columbia University Center for Public Health Preparedness, at the Mailman School of Public Health. He also holds an Adjunct Faculty appointment in The Rockefeller University. He was Program Manager for Biodefense at the federal Defense Advanced Research Projects Agency (DARPA), where he directed the Advanced Diagnostics program, co-directed the "Pathogen Countermeasures" program, and managed DARPA's research collaborations with Russian scientists. Before that, he was Assistant Professor (Virology), The Rockefeller University. He chaired the 1989 National Institutes of Health (NIH) Conference on Emerging Viruses, for which he originated the concept of "emerging viruses". Dr. Morse was founding Chair of ProMED (international Program to Monitor Emerging Diseases) and founding Section Editor of the CDC journal Emerging Infectious Diseases. He was also Secretary of the American Committee on Laboratory Animal Diseases (ACLAD). He is the editor of two books, *Emerging Viruses* (Oxford University Press, 1993; paperback, 1996), which was selected by American Scientist for its list of "The Top 100 Science Books of the [20th] Century", and *The Evolutionary Biology of Viruses* (Raven Press, 1994). He was a member of the IOM Committee on Emerging Microbial Threats to Health, IOM Committee on Xenograft Transplantation, and the NRC Committee on Animal Models for Testing Interventions Against Aerosolized Bioterrorism Agents. He served on the Steering Committee of the IOM Forum on Microbial Threats, in addition to serving on several other committees, government advisory panels, and journal editorial boards. He is a Fellow of the AAAS, the American Academy of Microbiology, the American College of Epidemiology, the New York Academy of Sciences (past Chair, Microbiology Section), the New York Academy of Medicine, and an elected life member of the Council on Foreign Relations.

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virus) and *Filoviridae* (Marburg and Ebola viruses), and elucidation of the pathology and pathogenesis of the diseases in man, monkeys, hamsters and guinea pigs caused by these exceptionally virulent agents; (4) Viral encephalitides: long running studies of the pathogenesis of neurotropic viruses in experimental animals, including alphaviruses, flaviviruses, bunyaviruses, enteroviruses, paramyxoviruses, herpesviruses, and others. He has been a leader in advancing the concept of "new and emerging infectious diseases" and "new and emerging zoonoses." Most recently his interests have included the threat posed by bioterrorism. Dr. Murphy has a B.S. in Bacteriology, a D.V.M. from Cornell University, and a Ph.D. in Comparative Pathology from UC Davis.

Vikram S Patel, PhD, is a Deputy Director in the Division of Drug Safety Research in CDER at FDA. He is responsible for guiding safety related preclinical research, including research in the area of toxicology, pharmacokinetics, drug metabolism and transporters. He is recognized for his expertise in pharmacokinetic/pharmacodynamic modeling and simulations, physiological modeling (including biomarker modeling and simulations), drug metabolism, in-vitro/-in vivo correlations, and in drug formulation and delivery. Prior to joining the FDA, Dr. Patel was Senior Director of Discovery Pharmacokinetics at Wyeth where he was responsible for overseeing and providing pharmacokinetic, Toxicokinetic, and pharmacokinetic/ pharmacodynamic support for all discovery projects (small and large molecules). Prior to 2002, he worked for Procter and Gamble Pharmaceuticals where he developed and established a GLP pre-clinical PK section. He also developed a sustained release product called Macrobid®, currently marketed worldwide. Dr. Patel has extensive experience in drug formulation, clinical and preclinical pharmacokinetic areas. His research interests include development of pharmacokinetic/pharmacodynamic models, modeling and characterization of absorptive processes, development of in vivo - in vitro relationships and use of pharmacokinetics in dosage form development and optimization.

James R. Swearengen, DVM, is the Comparative Medicine Veterinarian for the National Biodefense Analysis and Countermeasures Center and a former Senior Director of the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC International). Dr. Swearengen received his veterinary medical degree from the University of Missouri-Columbia in 1982. He is a former Deputy Commander of the U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland, and served as the laboratory animal medicine consultant to the U.S. Army Surgeon General. He retired from the U.S. Army in 2005. Dr. Swearengen is a member of the AAALAC International Council on Accreditation and is board certified in both veterinary preventive medicine and laboratory animal medicine. He is a Past-President of the American College of Laboratory Animal Medicine (ACLAM) and serves on the United States Animal Health Association Board of Directors.

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India Hook-Barnard, PhD, is Program Officer with the Board on Life Sciences of the National Research Council. She came to the National Academies from the National Institutes of Health where she was a Postdoctoral Research Fellow from 2003 to 2008. Her research investigating the molecular mechanism of gene expression focused on the interactions between RNA polymerase and promoter DNA. Dr. Hook-Barnard earned her PhD from the Dept. of Molecular Microbiology and Immunology at the University of Missouri. Her graduate research examined translational regulation and ribosome binding in Escherichia coli. At the National Academies, she contributes to projects in a variety of topic areas. Much of her current work is related to issues of molecular biology, microbiology, biosecurity, synthetic biology, and genomics. Dr. Hook-Barnard has directed the U.S. Canada Regional Committee for the International Brain Research Organization since 2008, and she was the study director for the consensus reports, *Sequence-Based Classification of Select Agents: A Brighter Line (2010)*; and *Toward Precision Medicine: Building a Knowledge Network for Biomedical Research and a New Taxonomy of Disease (2011)*.